EFFECT OF CULTIVAR AND ENVIRONMENT ON THE PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF PEA PROTEIN ISOLATES

A Thesis Submitted to the College of

Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Master of Science in the

Department of Food and Bioproduct Sciences

University of Saskatchewan

Saskatoon, Saskatchewan, Canada

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2016

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ABSTRACT

The overarching goal of this research was to investigate the effect of cultivar and environment on the physicochemical and functional properties of pea protein isolates using a structure-function approach. Six pea cultivars (Agassiz, CDC Golden, CDC Dakota, CDC Striker, CDC Tetris, Cooper) were collected from two years (2011, 2012) over two locations in Saskatchewan (Saskatoon and Rosthern) from two field replicates. Pea protein isolates were prepared from defatted flours by alkaline extraction (pH 9.0) followed by isoelectric precipitation (pH 4.5), and then neutralized to pH 7.0 prior to freeze-drying. Samples were evaluated for composition (amino acid profile, legumin/vicilin ratio), surface characteristics (zeta potential, surface hydrophobicity), and functional properties (nitrogen solubility, oil holding capacity, foaming capacity, foam stability, emulsion stability). In addition, samples were assessed for seed weight and colour, and compared against the functional characteristics of six commercially produced protein isolates (whey, wheat, egg, pea, and two soy ingredients).

The extracted pea protein isolates had protein contents of ~91% (d.b.), as well as isolate and protein yields of ~18% and ~72%, respectively. Although cultivars exhibited a range of legumin/vicilin ratios from 0.36 (Agassiz) to 0.79 (CDC Golden), such differences were not reflected in their amino acid profiles. Differences amongst cultivars, as well as significant cultivar × environment interactions, were found for only surface hydrophobicity (195-267 a.u.), solubility (63-75%), and foaming capacity (167-244%). No differences in either cultivar or environment were observed in other surface (zeta potential = ~-24 mV) or functional (oil holding capacity = ~3.2 g/g; foam stability = ~75%; emulsion stability = ~96%) properties. All functional properties were significantly correlated with legumin/vicilin ratio and/or surface hydrophobicity. However, such relationships were weak ($r = -0.19$ to -0.20, and $r = 0.17$ to 0.32). The strongest correlation was observed between the legumin/vicilin ratio and surface hydrophobicity at $r = 0.63$ for the pea protein isolates. Meanwhile, zeta potential did not display a significant correlation to any property tested.

In comparison to commercial protein isolates, the pea protein isolates behaved most similarly to soy except for solubility. Whey and egg were superior in solubility and the foaming properties, whereas wheat and the commercial pea protein product underperformed in almost all functionality tests. These findings suggest that while inherent protein material source may be

important to functional behaviours, the method of extraction could pose even greater effects. This was observed between the laboratory- and commercially-prepared pea protein isolates, which at minimum differed in processing (defatting) and method of drying (freeze- vs. spray-dried). Coupled with the weak correlations between physicochemical and functional properties, findings overall indicate that method of protein isolate production play a more significant role in protein functional characteristics than cultivar, environment, or composition. Findings also suggest that secondary processors may not need to specify either cultivar or environment of their raw materials, thus creating advantages in their feedstock sourcing.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to my supervisors Drs. Michael Nickerson and Robert Tyler for their constant guidance and encouragement, and delving out pieces of wisdom as I needed them. I would like to thank my advisory committee, Drs. Thomas Warkentin and Mehmet Tulbek, as well as past and present committee chairs, Drs. George Khachatourians and Xiao Qiu, for their invaluable insight and feedback. Thanks to Dr. Takuji Tanaka for acting as chair during my defense. I also wish to extend a special thank you to Dr. Lingyun Chen for serving as my external examiner.

I greatly appreciate all the assistance received during sample preparation and analysis from Christine Lim, Bushra Azmathallah, and the staff at POS Bio-Sciences, Crop Science Field Lab, and the Grains Innovation Laboratory. In addition, I would like to acknowledge Dilshan Benaragama and Marwan Diapari from the Department of Plant Sciences for going out of their ways in helping me with statistical analyses. I would also like to thank Paul Screpnek from the Saskatchewan Crop Insurance Corporation for locating and sharing environmental data for the field sites used in this project.

I would like to thank all of the faculty, staff, and students within the Department of Food and Bioproduct Sciences for their support, friendship, and insightful discussions. Thank you to Heather Silcox, Yuanlong Cao, Gerhard Maynard, and Shahram Emami for their technical assistance, and to the administrative support staff Ann Harley, Tanya Napper, Kendra Panko, Patricia Olesiuk, and Donna Selby for handling all my miscellaneous inquiries, and for making my day better with their friendliness and candy supply.

To my parents Yuk Won Chow and Kwok Ying Lam, and my sister Wendy Lam – a very special thank you goes out to them for expressing their love and support in their individually unique ways throughout this journey and all of my life.

I am deeply grateful to my Toastmasters family who, through a combination of force and encouragement, gave me the confidence to pursue a number of tasks I never would have imagined volunteering for.

Last but definitely not least, I would like to thank the Saskatchewan Agriculture Development Fund and Saskatchewan Pulse Growers for providing financial support for my research.

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LIST OF ABBREVIATIONS AND SYMBOLS

AAFC	Agriculture and Agri-Food Canada
a.u.	Arbitrary unit
CDC	Crop Development Centre
d.b.	Dry basis
EPI	Egg protein isolate
ES	Emulsion stability
EDTA	Ethylenediaminetetraacetic acid
FC	Foaming capacity
FS	Foaming stability
HPLC	High performance liquid chromatography
pI	Isoelectric point
IEP	Isoelectric precipitation
Lg	Legumin
O/W	Oil-in-water
OHC	Oil holding capacity
PPI	Pea protein isolate
PPIc	Pea protein isolate - commercial
PRVT	Provincial Regional Variety Trials
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SE	Salt extraction
SPI	Soy protein isolate
H_0	Surface hydrophobicity
UF	Ultrafiltration
Vn	Vicilin
W/O	Water-in-oil
WhPI	Wheat protein isolate
WPI	Whey protein isolate
ZP	Zeta potential
κ	Debye length

η	Dispersion viscosity
U_E	Electrophoretic mobility
g	Gravitational force
N	Nitrogen
ε	Permittivity
S	Svedberg unit (sedimentation coefficient)
ζ	Zeta potential (in an equation)

1. INTRODUCTION

1.1 Overview

Protein products derived from dairy, egg, soy, and wheat are commonly used by the food industry for both their nutritional and functional properties (Adebiyi & Aluko, 2011; Towes & Wang, 2013). However, protein markets are now seeing shifts away from these ingredients toward alternative sources (e.g., pea) due to consumers' perceived fears about consuming animal-derived products, dietary choices based on religious or moral preferences, allergenicity, and genetic modification (Can Karaca et al., 2011; Mertens et al., 2011; Toews & Wang, 2013). Overall, plant proteins (other than soy and wheat) remain relatively underutilized as food ingredients, where information on their structural and functional properties is limited (Adebiyi & Aluko, 2011).

Pea (*Pisum sativum* L.) is an important source of nutrition for both humans and animals. It is rich in protein (23-31%) containing high levels of arginine and lysine (Boye, et al., 2010b; Swanson, 1990). Pea is limiting in methionine and tryptophan, but has a higher lysine content than other legumes (Mertens et al., 2012). Accordingly, pea is especially complementary of cereal grains in fulfilling the essential amino acid profile, since cereals are generally deficient in lysine but have higher levels of sulfur amino acids (methionine, cysteine) than legumes (Adebiyi & Aluko, 2011; Mertens et al., 2011; Olson & Frey, 1987). Legume proteins are dominated by two classes of proteins, namely albumins and globulins, which comprise 10-20% and 70-80% of the total protein found within the seed (Can Karaca et al., 2011; Duranti & Scarafoni, 1999). Albumins are the water-soluble metabolic proteins in legume seeds, whereas globulins are the salt-soluble storage proteins. In pea, globulins are dominated by the 11S (legumin; 300-400 kDa) and 7S (vicilin; 150-180 kDa) fractions (Can Karaca et al., 2011). Legumin is a hexamer with disulfide-bonded α and β subunits, whereas vicilin is a trimer made of α, β, and γ subunits primarily held together by hydrophobic interactions (Mertens et al., 2011; Sikorski, 2001). The ratio of legumin to vicilin ranges from 0.4 to 2.0 and is dependent on a number of intrinsic and extrinsic factors (Mertens et al., 2011; Schroeder, 1982). Legumin and vicilin have different functional properties

due to their different amino acid compositions and structures. For example, vicilin possesses better gel-forming and emulsifying properties than does legumin (Barac et al., 2010).

This research will characterize the functional performance of protein isolates extracted from pea by its legumin/vicilin (Lg/Vn) ratio and surface properties, and examine factors that affect their composition and behaviours, such as cultivar, growing location, and year.

1.2 Objectives

The overall goal of this research was to evaluate the effect of cultivar and environment on the functionality of protein isolates prepared from pea, with the aim of identifying cultivars with better potential as food ingredients using a structure-function approach. The specific objectives were: (a) to evaluate the effect of cultivar and environment on the Lg/Vn ratio and surface properties of pea protein isolates; (b) to evaluate the effect of cultivar and environment on the functional properties of pea protein isolates; and (c) to determine the relationships between Lg/Vn ratio, surface characteristics, and functionality.

1.3 Hypotheses

The following hypotheses were tested in support of the overall goal of this research: (a) both the cultivar and the growing environment of pea will impact the physicochemical and functional characteristics of pea protein isolates; and (b) the functional behaviours of pea protein isolates will be dependent on the physicochemical properties of the proteins.

2. LITERATURE REVIEW

2.1 Pea protein structure

Depending on the variety, maturity at harvest, and growing conditions, field pea comprises 23.1-30.9% protein, 1.5-2.0% fat, and minor constituents such as vitamins, minerals, phytic acid, polyphenols, saponin, and oxalate (Boye et al., 2010b; Gueguen, 1983; Tiwari et al., 2011). Its carbohydrate content primarily of starch (35-40%; 24.0-49.0% amylose) and dietary fibre (10-15% insoluble and 2-9% soluble) ranges from 60-65%, which also includes non-starch polysaccharides such as sucrose, oligosaccharide, and cellulose (Hoover et al., 2010; Tiwari et al., 2011). Pea protein is dominated by two classes of proteins, namely albumins and globulins, representing 10-20% and 70-80% of the total proteins found within the seed, respectively (Can Karaca et al., 2011; Duranti & Scarafoni, 1999). Albumins are considered to be water-soluble metabolic proteins which, in pea, compared to globulins contain higher concentrations of the essential amino acids tryptophan, lysine, threonine, and methionine (Boye et al., 2010b). Globulins are considered to be salt-soluble storage proteins, and can be further sub-divided into mainly legumin and vicilin proteins, with minor amounts of a third known as convicilin.

Legumin is a hexameric protein with a molecular mass between 300-400 kDa and a sedimentation coefficient of 11S (Svedberg units). An acidic (40 kDa)-basic (20 kDa) (α-β) subunit covalently linked by a disulfide bond between cysteine residues represents one monomer within a non-covalently linked, quaternary structure (Mertens et al., 2011; Sikorski, 2001). Although each α- and β-chain shows some heterogeneity, the α-chain is dominated by glutamic acid and has leucine as the N-terminal amino group, whereas the β-chain contains more alanine, valine, and leucine and has glycine as the N-terminal amino group (Sikorski, 2001).

Vicilin proteins are trimers with a molecular mass of 150-170 kDa and sedimentation coefficient of 7S (Sikorski, 2001). Each monomer is ~47-50 kDa and consists of three subunits (α, β, and γ), providing two possible sites for post-translational proteolytic cleavage that results in fractions of 12 to 36 kDa (Gatehouse et al., 1982; Tzitzikas et al., 2006). In contrast to legumin, vicilin is held together by hydrophobic interactions rather than covalent disulfide bonds (Sikorski,

2011). Also, the γ-subunit is sometimes N-glycosylated near the C terminus, whereas glycosylation has not been verified in legumin (Swanson, 1990; Mession et al., 2012). Vicilin contains low levels of sulfur-containing amino acids (methionine, cysteine) and tryptophan, and higher levels of basic (arginine, lysine) and acidic (aspartic acid, glutamic acid) amino acids (Jackson et al., 1969; Sikorski, 2011). N-terminal amino groups typically are represented by serine, glutamic acid, and aspartic acid (Sikorski, 2001). Although amino acid fingerprinting showed 70-80% homogeneity between legumin and vicilin belonging to the tribe Vicieae (or Fabeae), N-terminal analysis suggested greater heterogeneity (Jackson et al., 1969). Both the legumin and vicilin proteins are dominated by β-sheet type secondary structures (Sikorski, 2001).

Convicilin is a third storage protein found in pea and other pulses and has a molecular mass of ~70 kDa. It can form trimers of ~210 kDa (or ~290 kDa including an N-terminal extension) with three convicilin molecules or heteromeric trimers with vicilin (Boye et al., 2010b; Tzitzikas et al., 2006). The amino acid profile of convicilin is distinct from both legumin and vicilin, and unlike vicilin, it contains sulfur-containing amino acids and a highly-charged N-terminal extension (Boulter, 1983; Boye et al., 2010b; Tzitzikas et al., 2006). Albumin proteins are water-soluble, have molecular masses ranging between 5 and 80 kDa, and comprise enzymes, protease inhibitors, amylase inhibitors, and lectins (Boye et al., 2010b). Prolamins (soluble in dilute alcohols) and glutenins (soluble in dilute acids) can also be found in minor amounts (Boye et al., 2010b).

2.2 Effect of cultivar and environment on pea protein content

In field pea breeding programs for both food and feed, the main priorities are cultivars with high yield, early maturation, and resistance to lodging and disease (Vera et al., 2000). Due to changes in the performance of pea in response to environmental conditions, such as soil type, rainfall, and temperature, potential pea cultivars should be tested at different locations over several years to determine the extent of environmental effects on genotypes (Acikgoz et al., 2009; Nikolopoulou et al., 2007; Wang et al., 2010).

The specific effects of the environmental conditions on pea genotype performance have been difficult to isolate. High temperatures and low rainfall have been associated with greater protein yield (Al-Karaki and Ereifej, 1999; Nikolopoulou et al., 2007). For example, Nikolopoulou et al. (2007) found that between two locations with a rainfall difference of 209 mm, pea seed grown in the drier location was, on average, 7% higher in protein. However, McLean et al. (1974)

observed only a 1.5% increase in protein content between plants grown under extreme moisture conditions of periodic wilting (10% moisture) and maximum water capacity (26% moisture). A negative correlation between protein content and seed yield (by weight) has been reported by authors conducting independent studies using different pea varieties grown in different locations and years (Sosulski et al., 1974; Al-Karaki and Ereifej, 1999; Wang et al., 2010).

Reichert and MacKenzie (1982) sampled dehulled pea (cultivar Trapper) grown in four locations in Saskatchewan, Canada, of which location was the suspected reason for inconsistent protein levels of 14.5%, 18.3%, 24.3%, and 28.5% (d.b.). They found that protein content had very strong negative correlations ($-0.91 < r < -0.99$) to starch, lipid, ash, soluble sugar, and neutral detergent fibre, where protein content variability was especially attributed to starch content. Cousin (1997) established starch synthesis as the determining factor of protein content in pea, by which he consistently observed higher protein content in wrinkled pea (26-33%) compared to smooth pea (23-31%), which are more abundant in starch.

2.3 Legumin/vicilin ratio

2.3.1 Legumin/vicilin ratio in pea

At maturity, the legumin/vicilin (Lg/Vn) ratio of field peas ranges from 0.4 to 2.0 (Schroeder, 1982). The Lg/Vn ratio increases throughout seed development due to different rates of synthesis of the 11S and 7S protein fractions. Vicilin synthesis is dominant from early development until seventeen days after flowering, whereas legumin is rapidly synthesized in the latter stages of development, from twenty days after flowering and onward (Chandler et al., 1984). Danielsson (1952) measured a Lg/Vn ratio change from 0.37 to 0.67 in field pea sampled twenty days apart, whereas Wright and Boulter (1972) reported a nine-fold increase in legumin, compared to a two-fold increase in vicilin, in faba bean between days 40 and 50 of growth.

Protein composition is dependent on a number of intrinsic and extrinsic factors. Cousin et al. (1992) observed that smooth pea varieties, which have lower protein content than wrinkled varieties, have a much higher Lg/Vn ratio. This, however, contradicts their overall observation that the Lg/Vn ratio increases with protein content (Swanson, 1990; Cousin et al., 1992). Legumin and vicilin are the most environmentally sensitive proteins in peas, and are highly susceptible to extrinsic factors such as agronomic practices, environmental conditions, and even the method used to detect protein composition (Mertens et al., 2011). When grown under sulfur-deficient

conditions, vicilin synthesis is maintained throughout development, whereas the synthesis of sulfur-rich legumin is greatly compromised or undetectable in severely deficient conditions (Chandler et al., 1984).

2.3.2 Methods of legumin/vicilin ratio determination

Several methods have been widely employed for the quantification of legumin and vicilin. Such methods utilize the molecular masses and chemical properties of the two fractions for their separation. In ultracentrifugation, protein fractions separate according to their sedimentation coefficients. Proteins are subject to a strong centrifugal field and a density gradient medium, where the concentration distribution of the rate of settling is measured by light absorption and refraction (Svedberg, 1937). Another method is differential scanning calorimetry, which differentiates protein fractions by their temperatures and enthalpies during denaturation (Chambers et al., 1992). Several immunological techniques based on the reaction between antigens and antibodies have been applied. Antibodies must first be raised in animals, such as rabbits, by subcutaneous injection of the protein followed by blood collection and purification of the antibodies (Casey, 1979). The complexes formed between these antibodies and proteins are then characterized, for example, in Laurell's rocket immunoelectrophoresis where proteins pass through an agarose gel containing antibodies and are analyzed against a standard (Laurell, 1966). Another common electrophoretic technique is polyacrylamide gel electrophoresis (PAGE), where proteins travel through an electric field at various rates, dependent on size, charge, and molecular shape (Nielsen, 2010). With the addition of sodium dodecyl sulfate (SDS), molecular shape and charges on proteins are negated to permit separation by size only. β-mercaptoethanol can be added to break disulfide bonds between proteins. Protein content is then quantified by densitometry, an optical absorption method. Lastly, separation by liquid column chromatography, such as high performance liquid chromatography (HPLC), is reflective of the affinity of a sample for a stationary phase while being carried in a mobile phase. It has multiple advantages over the aforementioned methods, namely good resolution and reproducibility, as well as being adaptable to any legume protein (Chambers et al., 1992).

2.4 Protein extraction methods

2.4.1 Alkaline extraction/isoelectric precipitation

Alkaline extraction followed by isoelectric precipitation is a wet process that utilizes the high solubility of legume proteins in alkaline conditions and minimal solubility at their isoelectric point (pI), between pH 4 and 5 (Boye et al., 2010b). This process takes advantage of the similar solubility characteristics of legumin and vicilin, and is the most common method of legume protein extraction reported in the literature (Gueguen, 1983; Hoang, 2012). Briefly, defatted legume flour, with or without seed coat, is dispersed in water, adjusted to an alkaline pH using sodium, potassium, or calcium hydroxide, and left to stand for 30 to 180 minutes to maximize protein solubility (Boye et al., 2010b; Gueguen, 1983). Without the defatting step, protein-lipid interactions limit protein solubility to reduce final yields obtained. The temperature might also be increased up to 50-60°C to aid solubilization (Anson & Pader, 1957; Hall, 1996). Higher temperatures tend to be avoided to limit protein denaturation. The mixture is then centrifuged, where the supernatant is collected and adjusted to the isoelectric pH using hydrochloric acid or sulfuric acid. The precipitated protein is collected by centrifugation, washed, neutralized, and dried by drum, spray, or freeze drying (Boye et al., 2010b; Gueguen, 1983).

Optimal processing conditions can produce isolate yields of 80% to 94%, but conditions used in each process can affect protein purity, yield, and functionality (Hoang, 2012). Hoang (2012) determined that the flour:water ratio and extraction pH were the most critical factors. Flour:water ratios of 1:5 up to 1:20 (w/v) have been reported (Boye et al., 2010b), but Hoang (2012) reasoned that the increased concentration gradient between the solid and liquid phases in a low ratio slurry can improve solubility. For extraction pH, although higher alkalinity results in greater solubility and protein yield, pH 11 and above is associated with increased starch swelling, which leads to starch contamination in the isolate product (Hoang, 2012). Alkaline extraction is also responsible for other adverse chemical reactions, such as the conversion of cysteine and serine residues to nephrotoxic lysinoalanine compounds, reduced bioavailability of proteins, and racemization of amino acids (Fabian & Ju, 2011; Swanson, 1990). Also, while processing conditions using highly alkaline pH, high temperature, and long standing times are associated with higher isolate yield, the isolate is susceptible to greater damage (i.e., protein denaturation) (Cone & Brown, 1934; Swanson, 1990).

Flour particle size and the type of solubilizing agent used also affect isolate yield. The optimal flour particle size for alkaline extraction is 100-150 μm, and sodium and potassium hydroxide have been found to generate better yields than calcium hydroxide (Owusu-Ansah et al., 1987). Lastly, a protein loss of 6.2% from the discarded supernatant is reported for this extraction method (Hoang, 2012).

2.4.2 Salt extraction and micellization

Salt extraction takes advantage of the salting in and out phenomena of proteins, followed by a desalting process to lower the ionic strength of the protein environment (Boye et al., 2010b; Murray et al., 1978). Briefly, flour is stirred for 10 to 60 minutes in a salt solution of specified ionic strength at a 1:10 (w/v) ratio, followed by removal of insoluble matter by settling, decanting, screening, filtering, or centrifuging. The supernatant is then desalted and dried (Boye et al., 2010b; Gueguen & Barbot, 1988; Murray et al., 1978). The concentration and choice of salt or mixture of salts are selected according to the salting in characteristics of the protein to be isolated, as well as the salting out characteristics of the unwanted proteins, since proteins precipitate at an array of ionic strengths (Berg et al., 2002; Jain, 1982). Other factors to consider include adverse interactions between the salt and sample components, and food grade safety (Ahmed, 2005; Murray et al., 1978). Generally, salting in of proteins occurs at low ionic strength, between 0.1 and 1 M (Hall, 1996).

Some advantages of salt extraction are that extreme alkaline or acidic pH, or elevated temperature, is not required. Extraction occurs at the natural pH level of the protein/water/salt mixture of 5.5 to 6.5, although Crévieu et al. (1996) suggested the use of a slightly alkaline pH to maximize protein solubility (Murray et al., 1978). The addition of acid or base might be needed to maintain the pH within this range, or a salt solution with buffering capacity may be used.

The high-salt protein extract supernatant should have a protein concentration of 15 mg/mL to 100 mg/mL (Murray et al., 1978). Several methods have been employed to decrease its ionic strength. In the micellization method, protein precipitation is induced by adding cold water at a ratio of 1:3 to 1:10 of high-salt protein extract to water (Murray et al., 1978; Mwasaru et al., 1999). Dilution of the protein solution forces solubilized proteins to adjust to the low ionic strength environment via a series of dissociation reactions to form loosely associated, lower molecular weight aggregates. Upon reaching a critical protein concentration, the aggregates re-associate into

a comparatively low molecular weight species called micelles and are precipitated out (Murray et al., 1978). Micelles are arranged as thermodynamically stable spheres with minimized interfacial energy by exposing polar moieties to the outer aqueous environment, whereas hydrophobic moieties gather toward the centre. Proteins with greater surface hydrophobicity increase protein-protein interactions and are more successful at creating large, uniform aggregates (Murray et al., 1978). The diluted solution may be left to stand for a time to maximize micelle formation. It is then centrifuged, where the high salt aqueous phase is discarded and the pellet is dried (Boye et al., 2010b; Murray et al., 1978). Using a 0.25 M NaCl solution at pH 6.5 and a micellization standing time of 6 hours, Mwasaru et al. (1999) found that pigeon pea yielded a product with a protein content of 40.2%, whereas cowpea yielded a product with 36.7% protein. These values are comparable to their alkaline-extracted samples at pH 10.5 and 8.5, respectively, where yields increased with alkalinity. Meanwhile, Gueguen (1983) reported that up to 95% yield might be attainable using the micellization method.

Another widely used method for desalting is dialysis. Dialysis is a membrane separation process. It is driven by a chemical potential gradient to diffuse water and low molecular weight solutes, such as salt, across a semi-permeable membrane (Jain, 1982). For pea proteins, Gueguen and Barbot (1988) and Crévieu et al. (1996) used membranes with cut-offs of 8,000 Da and 12,000-14,000 Da, respectively. Diffusion requires time for both sides to equilibrate and is complete when the chemical gradient becomes negligible (Jain, 1982). Multiple changes of fresh, pre-cooled liquid for the sample to be dialyzed against ensures that very low concentrations of solutes remain in the sample. For example, Gueguen and Barbot (1988) cited a 130-hour process that required five changes of water of twenty times the extract volume. Crévieu et al. (1996) dialyzed globulin solutions against two changes of ten times the extract volume of ammonium carbonate, which required 70 hours and resulted in a 66.8% yield. Dialysis is also useful for separating albumin and globulin fractions. According to the Osborne protein classification, centrifugation of the dialyzed sample results in dissolved albumin fractions in the supernatant and precipitated globulin fractions in the pellet (Gueguen & Barbot, 1988).

2.5 Protein functionality

2.5.1 Solubility

Protein solubility can be defined as the equilibrium between protein-protein (hydrophobic) and protein-solvent (hydrophilic) interactions, expressed as Protein-Solvent ↔ Protein-Protein + Solvent-Solvent (Hall, 1996). Other definitions include the ratio of protein present in the liquid phase to protein present in both the liquid and solid phases under thermodynamic equilibrium conditions, or the retention of proteins in the supernatant after centrifugation (Hall, 1996). The solvent in most cases is usually water or buffer. The main determinant of protein solubility is the proportion and distribution of hydrophilic and hydrophobic groups on the surface of the molecule (Hall, 1996). In water, hydrophilic amino acid residues tend to orient toward the solvent interface, whereas a majority of the hydrophobic residues are buried in the interior of the protein to minimize free energy. Hydrophobic residues that remain on the protein surface create hydrophobic patches that hinder solubility. Solvent pH, ionic strength, temperature, and organic solvent components are also major factors influencing solubility (Damodaran et al., 2008).

Protein solubility is sometimes referred to as "nitrogen solubility", since nitrogen from both protein and non-protein sources, such as nucleic acids, free amino acids, peptides, and phospholipids, are extracted in solubility tests (Smith et al., 1966). Nitrogen solubility can be defined as the ratio of water-soluble nitrogen to total nitrogen, expressed as a percentage (Smith et al., 1966). The term "protein dispersibility" is also sometimes used, which refers to how easily powder aggregates are able to come apart and disperse uniformly in water (Hall, 1996).

At pHs above and below the pI, solubility is increased due to electrostatic repulsion brought on by positive and negative net charges on the protein surface (Hall, 1996). A protein exhibits lowest solubility at its isoelectric pH since it carries a zero net charge, minimizing electrostatic repulsive forces. Under these conditions, hydrophobic interactions between neighboring proteins can lead to aggregation and once aggregates are sufficient in size and number, precipitation occurs (Hall, 1996). In general, pea protein isolates exhibit lowest solubility between pH 4 to 6 irrespective of extraction method or pea cultivar (Boye et al., 2010a; Taherian et al., 2011; Withana-Gamage et al., 2011). Alebiyi and Aluko (2011) described a commercial pea protein isolate (Nutri-Pea Ltd., Portage la Prairie, MB, Canada) which demonstrated poor solubility, where maximum values of 30% were reached at pH 8. Using a pea protein isolate obtained from the same manufacturer, Taherian et al. (2011) found that it displayed a similar pattern of solubility from pH

2 to 9 as a ultrafiltration-extracted (UF) Eclipe pea isolate (yellow market class), but at significantly lower values. They suggested that hydrophobic legumin might have become exposed during processing, which resulted in increased surface hydrophobicity of the product. Can Karaca et al. (2011) observed a negative correlation between solubility and surface hydrophobicity, as well as a positive correlation between solubility and surface charge. These authors reported that at pH 7, pea protein isolates prepared from the CDC Leroy cultivar (forage market class) had solubilities of 61.4% and 38.1% when extracted by isoelectric precipitation (IEP) and salt extraction (SE), respectively. Pea presented amongst the lowest solubility between kabuli chickpea, faba bean, lentil, and soy isolates, in addition to high surface hydrophobicity and low surface charge. Boye et al. (2010a) reported that a pea protein isolate sourced from the CDC Golden cultivar (yellow market class) displayed highest solubility at pHs 1 and 7, compared to isolates from red and green lentils, and desi and kabuli chickpeas. Inconsistent differences were noted for pea protein isolates extracted by IEP and UF, where IEP resulted in solubility of 90% at pH 1, but 29% at pH 3, whereas UF isolates had solubilities of 60% and 56%, respectively. Vose (1980) also reported inconsistencies for protein isolates prepared from Trapper (forage market class) pea, where IEP isolates exhibited 66% solubility at pH 3 and 7. At these pH levels, while the UF prepared isolate displayed lower solubility, it exhibited 15% higher solubility overall between pHs 2 and 10.

The presence of salts in solution can act to screen the electric double layer (i.e., diffuse and stern layers) surrounding the protein, effectively reducing the zeta potential and the amount of electrostatic repulsive forces occurring. Consequently, proteins behave as if they have reduced or low net charge and aggregate via hydrophobic interactions. Once aggregates are sufficient in size and number, precipitation of the protein occurs. Depending on the type and concentration of salt present, proteins may undergo ‘salting in’ or ‘salting out’. In the case of the former, thiocyanate, perchlorate, barium, and calcium salts promote protein-water interactions and ordering of hydration layers surrounding the protein to increase solubility (Damodaran et al., 2008; Hall, 1996; Walstra, 2003). In contrast, sulfate, hydrogen phosphate, ammonium, and potassium salts promote ion-water interactions, which act to disrupt the hydration layers surrounding the proteins to cause exposure of hydrophobic moieties (Damodaran et al., 2008; Hall, 1996; Walstra, 2003). Consequently, aggregation and precipitation ensues, depending on the ionic strength and level of hydrophobicity.

Generally, protein solubilization increases as the temperature is raised from 0°C to 50°C, up to a temperature where non-covalent bonds (e.g., hydrogen bonds) become destabilized and secondary and tertiary structures are lost (Hall, 1996). Protein denaturation induces interaction between hydrophobic groups, which leads to precipitation and decreased solubility. However, Walstra (2003) stated that hydrophobic interactions increase greatly between 0°C and 60°C. In contrast, organic solvents such as acetone lower the dielectric constant of the solvent medium, where the dielectric constant is defined as an index of resistance to an electric current passing through a sample. This unfolds protein molecules through increased repulsive, intramolecular electrostatic forces, and promotes intermolecular electrostatic forces between oppositely charged groups, resulting in precipitation (Damodaran et al, 2008; Nielsen, 2010).

2.5.2 Oil holding capacity

Oil holding capacity (OHC), or oil absorption capacity, is defined as the amount of oil that can be absorbed per gram of protein (Lin & Zayas, 1987). Lipids and proteins interact through the binding of the aliphatic chains of lipid to the non-polar side chains of amino acids; therefore, proteins with higher hydrophobicity tend to have a greater propensity to hold oils (Sanjeewa, 2008; Withana-Gamage et al., 2011). OHC values can be influenced by the matrix structure of a protein, the type of lipid present, and the distribution and stability of lipids. The latter is affected by both droplet size and distribution, and the presence of emulsifying agents (Hall, 1996). Reported OHC values for pulse isolates are quite variable, and relate to, amongst other things, the type and variety of pulse and the processing conditions used to prepare the isolate (Boye et al., 2010a). Using isolates of Miranda yellow pea precipitated by acid, magnesium, or calcium via thirteen pH and temperature combinations, Soetrisno and Holmes (1992) found OHC to be consistently lower as extraction temperature was decreased. They also found that the interaction of high pH and temperature decreased OHC for both magnesium- and calcium-precipitated isolates, whereas the interaction of low pH and temperature affected only magnesium precipitation. This suggests that the choice of precipitating agent can affect the OHC of salt-extracted protein isolates; however, no possible mechanism was proposed. The highest OHC values were 5.22 g/g and 5.10 g/g for magnesium- and calcium-precipitated pea protein isolates, respectively. An IEP pea protein isolate (cultivar undisclosed) was reported by Withana-Gamage et al. (2011) to have an OHC value of 2.70 g/g, which was much lower than isolates from kabuli and desi chickpea (3.06-5.74 g/g).

Meanwhile, Boye et al. (2010b) reported OHC of 1.20 g/g for a pea protein isolate prepared by IEP, which was between faba bean (1.60 g/g) and soy (1.10 g/g) protein isolates in terms of magnitude. The OHC of commercially available isolates from smooth pea (Pisane HD, Cosucra) and soybean (Soyamin 90, Lucas Meyer Ltd.) were reported to be 1.59 g/g and 1.23 g/g, respectively (Fuhrmeister & Meuser, 2003). Boye et al. (2010a) conveyed that OHC values of isolates from yellow pea (CDC Golden), red and green lentil, and kabuli and desi chickpea were similar when isolates were prepared by IEP. However, red lentil and yellow pea had the greatest OHC at 2.26 g/g and 1.77 g/g, respectively, when prepared by UF. Fuhrmeister and Meuser (2003) found that a wrinkled pea isolate prepared by UF had an OHC of 1.32 g/g, whereas the OHC of isolates precipitated by acid, heat, or acid-heat treatments did not exceed 0.87 g/g. Meanwhile, the OHC of an acid-precipitated Miranda yellow pea isolate was 5.34 g/g, which Soetrisno and Holmes (1992) attributed to exposure of hydrophobic groups during denaturation in the extraction process.

2.5.3 Emulsification

An emulsion is the dispersion or suspension of two immiscible liquids created by mechanical agitation, resulting in a dispersed phase of submicron droplets suspended within a continuous phase (Hall, 1996). In foods, emulsions are of either oil-in-water (O/W) type, such as milk and mayonnaise, or water-in-oil (W/O) type, such as butter and margarine (Walstra, 2003). Emulsions are thermodynamically unstable because such an arrangement increases the interfacial area, thereby increasing the interfacial free energy of the system. Over time, O/W emulsions are prone to the phenomena of creaming, flocculation, and coalescence as the system attempts to minimize its free energy (Walstra, 2003). Creaming is the reversible rise of dispersed droplets to the surface against gravity due to density differences (0.05 g/cm^3 for most food grade oils) between the two phases (Damodaran, 2005). Flocculation is the reversible or irreversible aggregation of dispersed droplets due to an imbalance of attractive (van der Waals) and repulsive (electrostatic and steric) forces. Coalescence occurs when the continuous phase film separating the dispersed phase is ruptured, resulting in the irreversible merging of individual dispersed droplets into larger droplets (Damodaran, 2005; Dickinson, 2010).

Proteins adsorb to the interface to minimize the interfacial tension between the two phases. They align at the interface according to their amphiphilic nature and conform to train, loop, and tail configurations to form a viscoelastic interfacial film. Trains lie along the interface while loops

and tails protrude into the continuous phase to facilitate repulsion (Damodaran, 2005). Proteins differ in the minimum amount required for monolayer coverage of droplets and in the rate of adsorption to the oil-water interface. These factors, along with homogenizer energy output, determine droplet size, of which smaller radii confer a more stable emulsion (McClements, 2004). The net charge of a protein and its ability to rapidly re-orient to the interface determine its molecular flexibility, which is cited as the most important characteristic of a good emulsifier (Damodaran, 2005). Globular proteins are less flexible and require more time to align to the interface (McClements, 2004). Emulsions are more stable away from the pI of a protein and at low ionic strength. At pHs away from the pI, because the dispersed droplets are farther apart, the interactions between proteins adsorbed to different droplets are weakened. This in turn might promote stronger interactions between proteins adsorbed to the same droplet to form a robust interfacial film and deter coalescence (McClements, 2004). Likewise, emulsions are least stable close to the pI of a protein and at high ionic strength, because the dispersed phase is in close proximity and electrostatic repulsion is weak relative to attractive forces between droplets (McClements, 2004). Instability is also promoted by low temperature since crystallized water molecules force dispersed droplets closer together, as well as by uneven emulsifier distribution on the droplet surface (McClements, 2004). Meanwhile, partially denatured proteins and the use of more polar oils can improve stability, since hydrophobic groups are exposed and less unravelling of proteins is necessary (Damodaran, 2005; McClements, 2004).

Emulsion properties have been measured using many methods. A common method is by the determination of the emulsion activity index (EAI), which estimates the interfacial area that can be stabilized per unit weight of protein, or by the determination of the emulsion stability index (ESI), which measures the ability of an emulstion to resist changes over time (Boye et al., 2010b, Can Karaca et al., 2011). Similar to ESI, emulsion stability (ES) is the percentage of an emulsion which has not succumbed to separation (as a serum layer) after a specified length of time (Liu et al., 2010). Emulsion capacity (EC) is a measure of the maximum amount of oil that can be emulsified per unit weight of protein, before the O/W emulsion reaches its inversion point and becomes a W/O emulsion, as signified by a large drop in conductivity (Can Karaca et al, 2011; Hall, 1996).

Measurement techniques varied among authors and values were reported using various units, making comparison difficult. Using IEP and SE isolates from several legumes, Can Karaca

et al. (2011) found that EC was significantly affected by extraction method, but not by the legume source when tested with isolates from chickpea, faba bean, lentil, pea, and soy. At pH 7.0, an IEP yellow pea (CDC Leroy) isolate had an EC of 477.78 g oil/g protein, whereas a SE isolate yielded a value of 484.45 g oil/g protein. These authors reported that EC values for legume increased when surface charge and solubility increased, and surface hydrophobicity decreased. They also found that EAI, ESI, creaming stability, and mean droplet size were all significantly affected by the extraction method, legume source, and the interaction of these two factors, where IEP isolates yielded higher values overall with smaller droplet size. However, Boye et al. (2010a) proposed that EAI and ESI were affected only by legume source for isolates prepared from yellow pea, desi and kabuli chickpea, and green and red lentil. It is unknown whether different pea varieties would also display significance. Boye et al. (2010a) reported that both IEP and UF isolates of a yellow pea (CDC Golden) had nearly identical EAIs of 4.6 m^2/g, whereas Withana-Gamage et al. (2011) reported 0.7 m^2/g for an isolate from an unknown variety of pea protein isolate. Acid-precipitated wrinkled pea isolates yielded EAIs of 10.1 m^2/g and 14.0 m^2/g at pH 3.4 and 4, respectively (Boye et al., 2010a).

Can Karaca et al. (2011) stated that EAI is positively correlated to surface charge and solubility, and reported values of 42.9 m^2/g and 42.7 m^2/g for IEP and SE pea protein isolates, respectively, at pH 7.0. The same authors found ESI to be positively correlated to surface charge and solubility at pH 7.0, with values of 12.4 min and 10.9 min for IEP and SE pea protein isolates, respectively. Using a commercial pea protein isolate (Nutri-Pea Ltd.), Adebiyi and Aluko (2011) reported higher ESI values at neutral and alkaline pHs compared to acidic pH, possibly due to greater cohesiveness of interfacial proteins. Other ESI values include 19 min for both IEP and UF isolates from CDC Golden pea, and 18 min for an IEP pea protein isolate of unknown origin (Boye et al., 2010a; Withana-Gamage et al., 2011). Adebiyi and Aluko (2011) found droplet size to be reduced at neutral and alkaline pHs. They proposed that decreased solubility and a more folded protein structure at acidic pH led to lower molecular flexibility. In a previous study, Aluko et al. (2009) reported an oil droplet size range of 14 to 15 μm using a commercial yellow pea isolate (Nutri-Pea Ltd.) prepared by IEP. They found that a higher protein concentration led to smaller droplet size at pH 3, but not at isoelectric or neutral pH.

2.5.4 Foaming

Foams are dispersions of gas bubbles within a liquid (usually water) or solid continuous phase, and can be generated by sparging (forcing gas into the liquid phase through an aperture), whipping (beating atmospheric air into the liquid phase), shaking, or pouring (such as a glass of beer) (Hall, 1996). Due to high free energy at the gas-liquid interface, foams are thermodynamically unstable and undergo coalescence and disproportionation to reduce the interfacial area (Dickinson, 2010). Disproportionation, or Oswald ripening, is the diffusion of gas from small to large bubbles due to higher pressure within the former (Wierenga & Gruppen, 2010). Solubilized proteins diffuse and adsorb to the gas-liquid interface which reduces surface tension. They then unfold and orient hydrophobic regions to the gas phase and hydrophilic regions to the liquid phase to assume train and loop formations. A cohesive, continuous film is then formed around gas bubbles due to interactions between polypeptides (Kinsella, 1981; Wierenga & Gruppen, 2010). Ideally, the protein should adsorb rapidly to the gas-liquid interface and possess high molecular flexibility for quick reorientation. Newly formed bubbles tend to burst instantaneously due to the high surface tension in water. Accordingly, the foam volume – or capacity – is dependent on how quickly new air cells are formed and stabilized relative to the rate of collapse (Kinsella, 1981). Foaming capacity (FC) is the amount of interfacial area that can be created by the protein (Damodaran et al., 2008). It is positively correlated to the average hydrophobicity (difference in the free energy of amino acid side chains when exposed to a nonpolar solvent or water) of proteins, and can be enhanced by partial denaturation to increase surface activity (Damodaran, 2005; Kinsella, 1981). Because average hydrophobicity is derived using all amino acids in a protein, as opposed to only those exposed to the surface in surface hydrophobicity, the correlation of a functional property to average hydrophobicity suggests that the proteins exist in a more unfolded state to expose amino acids buried in the core (Damodaran, 2005). Boye et al. (2010a) reported the FC of isolates prepared from CDC Golden yellow pea to range from 95-105% when prepared by IEP or UF.

Foam stability (FS) is the ability of a protein to stabilize a foam against stresses (Damodaran et al., 2008). Stable foams tend to be resistant to gas diffusion, drainage and thinning of lamella fluid, and mechanical shock. Accordingly, stable protein-based foams should possess interfacial films that are cohesive through hydrogen bonding and electrostatic and hydrophobic interactions. Intermolecular associations should result in a network structure of high surface

elasticity to allow for some deformation (Kinsella, 1981; Wierenga & Gruppen, 2010). Unlike emulsions in which stability is induced by repulsion between the dispersed phase at pHs away from the pI, foams are most stable at the isoelectric pH of a protein. Because of minimal electrostatic repulsion, protein-protein interactions and adsorption to the interface are maximized, which promotes viscous film formation and steric stabilization. Boye et al. (2010a) reported that ~40% of the liquid remained in a foam after 5 min when stabilized by a protein isolate prepared from CDC Golden yellow pea, whether prepared by IEP or UF. Adebiyi and Aluko (2011) found that for a commercial pea protein isolate (Nutri-Pea Ltd.), FS steadily increased when tested at pH 4, 7, and 9, from about 30% to 80%. They predicted that foams were stabilized by electrostatic repulsion, which increased with pH and charge density. Wierenga and Gruppen (2010) reported that upon diffusion to the interface, proteins are less likely to adsorb as coverage approaches a maximum. Because protein-based foams can be formed at millimolar concentrations, excess proteins continually exchange between the continuous phase and interface, thus intermolecular associations gradually increase with aging to form a more cohesive film (Foegeding & Davis, 2011; Kinsella, 1981). Likewise, foaming capacity and foam stability usually improve at higher protein concentrations (Kinsella, 1981). Aluko et al. (2009), using an IEP commercial yellow pea protein isolate (Nutri-Pea Ltd.), found that FC increased with protein concentration up to 50 mg/mL at pH 3, but decreased at pHs 5 and 7. For all pH levels, FC decreased to between 50 and 120% when protein concentration was increased to 100 mg/mL, whereas values above 200% were possible at other concentrations and pHs. This was possibly due to limited solubility.

The addition of sugars has also been shown to improve FS by increasing lamella fluid viscosity to hinder drainage; however FC is impaired (Damodaran, 2005; Kinsella, 1981). Foaming properties are also enhanced when proteins are salted out in a salt solution, but impaired by the presence of lipids which adsorb more readily to the gas-fluid interface than proteins due to higher surface activity (Damodaran et al., 2008). Using UF Eclipse yellow pea protein isolate, Taherian et al. (2011) observed enhanced FS when NaCl was added up to 0.25%, with no improvement with a further increase in salt concentration. Greater stability was attributed to improved solubility and arrangement of proteins at the interface.

2.5.5 Functional properties of legumin and vicilin fractions

Due to its extensive quaternary structure and disulfide bridges, legumin has a more rigid conformation compared to vicilin (Dagorn-Scaviner et al., 1986). Accordingly, isolated vicilin fractions have demonstrated better functional behaviours than isolated legumin and mixed globulin fractions. Using SE fractions from B-160 green pea, Koyoro and Powers (1987) found that foams of isolated legumin and vicilin would not stabilize at pH 7 unless solutions were held at 90°C for 5 min. While both fractions had similar FC, legumin displayed lower FS despite having ~150% higher surface hydrophobicity than vicilin. This was attributed to the more flexible structure of vicilin. Dagorn-Scaviner et al. (1986) measured a lower energy barrier for vicilin to overcome when penetrating the foam surface film, but a much higher energy barrier than legumin during molecular conformation rearrangement within the adsorbed layer. The authors reasoned that vicilin might have undergone a more extensive molecular rearrangement and re-orientation process than legumin.

Better emulsifying properties of vicilin over legumin were also attributed to structural flexibility. Cserhalmi et al. (1998) tested SE fractions from five pea varieties, and observed better EAI and ESI for vicilin than for legumin overall. The authors warned that surface hydrophobicity, although also higher in vicilin, should not be used as a predictor for emulsifying behaviour due to the effect of pea variety. Following a literature review, Boye et al. (2010b) reported that better emulsifying properties in vicilin over legumin had been witnessed by other authors. Koyoro and Powers (1987) reported opposing results, where at pH 7, legumin displayed greater EC than vicilin, but similar ES was found for both fractions.

The pI of legumin was found to be at pH 4.8 (α-chain: pH 4.5-4.9; β-chain: pH 8.4-8.8), and at pH 5.5 for vicilin (Derbyshire et al., 1976; Krishna et al., 1979). Koyoro and Powers (1987) reported zero solubility for legumin and vicilin between pH 5 and 6 for a SE pea protein isolate. However, vicilin displayed better solubility at pH 7 (97%) compared to legumin (74%) and mixed globulin fractions (86%). Kimura et al. (2008) asserted that both N-terminal extensions and carbohydrate moieties in N-glycosylation contribute significantly to solubility at neutral and weak alkaline conditions, but N-glycosylation has not been confirmed for legumin (Swanson, 1990). Lastly, isolated vicilin fractions can undergo heat-induced gelation, whereas the ability of legumin to form a gel varies according to pea cultivar (Koyoro and Powers, 1987; Barac et al., 2010).

3. MATERIALS AND METHODS

3.1 Materials

Samples of six pea (*Pisum sativum* L.) cultivars (yellow cotyledon: CDC Golden, Agassiz, CDC Dakota; green cotyledon: CDC Striker, Cooper, CDC Tetris), sourced from the Saskatchewan regional pea variety trials (Rosthern, SK and the Sutherland field site near Saskatoon, SK) for the years 2011 and 2012, were provided by the Crop Development Centre, University of Saskatchewan (Saskatoon, SK, Canada) (Table 3.1). Two field replicates were used from each site-year (n = 48).

All chemicals used in this study, unless otherwise stated, were purchased from Sigma-Aldrich (Oakville, ON, Canada). Milli-Q™ water (EMD Millipore, Billerica, MA, USA) was used throughout the study.

Commercial products used in this study, including whey protein isolate (BiPRO JE, Davisco Foods International, Inc., Le Sueure, MN, USA), egg protein isolate (Dried Egg Whites Type H-40, Ballas Egg Products Corp., Zanesville, OH, USA), wheat protein isolate (Prolite® 100, Archer Daniels Midland Co. (ADM), Decatur, IL, USA), two soy protein isolates (Prolisse ISE-221, Cargill Health & Food Technologies, Wayzata, MN, USA; PRO-FAM 974®, ADM, Decatur, IL, USA), and pea protein isolate (Propulse, Nutri-Pea Limited, Portage la Prairie, MB), were kindly donated for this project.

3.2 Environments

Four environments were derived from the combination of two locations in Saskatchewan (Canada) and two years – Saskatoon 2011, Saskatoon 2012, Rosthern 2011, and Rosthern 2012. Environments were characterized by monthly total precipitation (mm) (Table 3.2) and monthly mean temperature (°C) (Table 3.3) during the growing season from May to August. Rosthern (black Chernozem soil zone) is located approximately 70 km north of Saskatoon (dark brown Chernozem soil zone).

Table 3.1. Summary of the characteristics and origin of six pea cultivars. All seed was sourced from the 2011 and 2012 Saskatchewan Provincial Regional Variety Trials. Samples (two field replicates) were obtained from each of two locations – Saskatoon, SK (Sutherland field site) and Rosthern, SK.

Cultivar	Cotyledon colour	Seed coat colour	Market class	Breeding institution	Year of release	Pedigree
CDC Golden	yellow	non-pigmented	yellow	CDC	2003	Local Syrian 1690/Alfetta//CDC Vienna/Express
Agassiz	yellow	non-pigmented	yellow	AAFC (Lacombe, AB; Morden, MB)	2006	MP1392/Grande
CDC Dakota	yellow	tan	dun	CDC	2010	DS Admiral/CDC Dundurn
CDC Striker	green	non-pigmented	green	CDC	2002	Majoret/P28RS-281
Cooper	green	non-pigmented	green	Limagrain (Netherlands)	2004	Baccara/92585
CDC Tetris	green	non-pigmented	green	CDC	2010	646-46/Escape

Table 3.2. Total precipitation by month for the 2011 and 2012 growing seasons at Saskatoon, SK and Rosthern, SK (Weather Innovations, 2014).

Month	Precipitation (mm)			
	Saskatoon		Rosthern	
	2011	2012	2011	2012
May	17.5	118.5	13.2	109
June	94.8	121.1	209.8	123
July	71.5	80.9	144.8	135
August	18.1	48.5	52.2	71.8
Total	201.9	369	420	439

Table 3.3 Average temperature by month for the 2011 and 2012 growing seasons at Saskatoon, SK and Rosthern, SK (Weather Innovations, 2014).

Month	Temperature (°C)			
	Saskatoon		Rosthern	
	2011	2012	2011	2012
May	10.9	10.1	10.7	9.9
June	15.5	15.8	15.4	15.5
July	18.4	19.7	17.6	19.1
August	17.2	17.3	16.8	17.1

3.3 Thousand seed weight

A seed counter (Agriculex ESC-1 electronic seed counter, Agriculex Inc., Guelph, Canada) was used to calculate the 1000-seed weights of non-dehulled whole peas, which were reported as grams per 1000 seeds.

3.4 Preparation of pea protein isolates

Samples of whole peas were dehulled using a Satake Grain Testing Mill (Satake Engineering Co., Ltd., Hiroshima, Japan) and cleaned of loose hull by aspiration (Ames Powercount Co., Brookings, SD, USA). Dehulled samples were first coarse-milled with a S500 Disc Mill (Glen Mills, Inc., Clifton, NJ, USA) at the finest setting possible (3.75), and then fine-

milled with a Cyclone Sample Mill (UDY Corp., Fort Collins, CO, USA) fitted with a 1-mm screen. Flours were defatted using a modification of the method of L'Hocine et al. (2006). In brief, flours were stirred in hexane (1:3, w/v) for 10 min and then vacuum-filtered through No. 1 Whatman filter paper (Whatman International Ltd., Maidstone, UK). The extraction process was repeated two times. Extracted samples were desolventized overnight in a fumehood. Protein isolates were prepared by alkaline extraction and isoelectric precipitation according to Can Karaca et al. (2011) with minor modifications. Flours were dispersed in water (1:10, w/v), adjusted to pH 9.0 using 1.0 M NaOH, stirred for 1 h at room temperature (22-23°C), and then centrifuged for 10 min at 4500 x *g* at 4°C (Sorvall RC-6 Plus centrifuge, Thermo Scientific, Ashville, NC, USA). Supernatants were collected and adjusted to pH 4.5 using 1.0 M HCl to precipitate protein. After centrifuging for 10 min at 4500 x *g* at 4°C, the pellets were rinsed, collected, liquefied using 150 mL water, and neutralized to pH 7.0 using 1.0 M NaOH. Protein isolates were stored at -30°C until freeze-dried (Labconco FreeZone 6 freeze-dryer, Labconco Corp., Kansas City, MO, USA), and then kept at 4 °C for long-term storage.

3.5 Compositional analysis

The composition (moisture, protein, ash, total lipid, and crude fat) of pea flours, pea protein isolates, and commercial protein isolates were determined in duplicate and reported on a moisture-free basis. The methods used are described in brief below.

3.5.1 Protein content

Protein contents of pea flours and pea protein isolates were determined by Official Method 990.03 of AOAC International (AOAC, 2005) using a FP-528PC Protein/Nitrogen Analyzer (LECO Corp., St. Joseph, MI, USA). Approximately 0.1 g of protein isolate or 0.2 g of flour was weighed into Quik-Cap Capsules (LECO Corp.) and combusted in the presence of oxygen. Nitrogen was then separated from other products through a chromatographic column and quantified. A nitrogen-to-protein conversion factor of 6.25 was used to calculate protein content. The analyzer was calibrated daily with EDTA and blanks, and accuracy was maintained by analyzing samples of a wheat flour of known protein content (%N × 5.70) for every 20 pea samples tested.

Protein concentrations in the commercial isolate products were determined at POS Bio-Sciences (Saskatoon, SK, Canada) by combustion (%N × 5.70 – wheat; %N × 6.25 – egg, soy, and pea; %N × 6.38 – whey) according to Official Method Ba 4e-93 of the American Oil Chemists' Society (AOCS, 1998).

3.5.2 Moisture

Moisture contents of pea flours and pea protein isolates were determined gravimetrically using a gravity-flow convection oven (Fisher Scientific™ Isotemp™ Standard Lab Oven, Thermo Fisher Scientific Inc., Waltham, MA, USA) set to 100-102°C according to a modification of Official Method 925.10 of AOAC International (AOAC, 2005). Aluminum dishes (57 mm) were dried for 1 h in the oven and then cooled for 1 h to room temperature in a desiccator. Samples (~0.5 g for protein isolates and ~1 g for flours) were weighed into pre-dried dishes, dried overnight in the oven, cooled for 1 h to room temperature in a desiccator, and then weighed using an analytical balance. Moisture content (%) was calculated using the following equation:

$$Moisture\ (\%) = \frac{wt\ of\ sample - wt\ of\ dried\ sample}{wt\ of\ sample} \times 100\% \quad \text{(eq. 1)}$$

For commercial protein isolates, moisture was determined at POS Bio-Sciences using Official Method Ba 2a-38 of the American Oil Chemists' Society (AOCS, 1998).

3.5.3 Ash

For pea flours and pea protein isolates, ash was determined gravimetrically using a muffle furnace (Fisher Scientific™ Isotemp™ Basic Muffle Furnace, Thermo Fisher Scientific Inc., Waltham, MA, USA) according to a modification of Official Method 923.03 of AOAC International (AOAC, 2005). Porcelain crucibles, fitted with lids, were pre-dried for 1 h at 550°C and then cooled to ~150°C (~2 h) before transfer to a glass desiccator to cool to room temperature (~1 h). Samples of approximately 0.5 g were weighed into pre-dried crucibles, charred on a hot plate set to high heat in a fumehood until fully blackened, and then ashed overnight at 550°C to obtain a white ash. Samples were cooled to ~150°C (~2 h) and then transferred to a glass desiccator for 1 h to cool to room temperature. The ash content (%) was calculated as follows:

$$Ash\ (\%) = \frac{wt\ of\ ash}{wt\ of\ sample} \times 100\%$$

(eq. 2)

Ash contents of commercial protein isolates were determined at POS Bio-Sciences using Official Method Bc 5-49 of the American Oil Chemists' Society (AOCS, 1998).

3.5.4 Lipid

Crude fat was determined for pea flours, pea protein isolates, and commercial protein isolates gravimetrically by Swedish tube extraction using petroleum ether at POS Bio-Sciences, according to the method of Troëng (1955). For pea flours and pea protein isolates, values were obtained for one field replicate of all site-years.

The polar lipid contents of pea protein isolates prepared from one field replicate from Saskatoon (2012) and of the commercial protein isolate samples were determined at POS Bio-Sciences using Official Method Ja 7b-91 of the American Oil Chemists' Society (AOCS, 1998). Total lipid levels were calculated as the sum of polar lipids and crude fat. For pea protein isolates, the mean crude fat values determined for each cultivar across all site-years were employed in the total lipid calculations.

3.6 Amino acid analysis

The amino acid compositions of pea flours and pea protein isolates from one field replicate from Rosthern (2011) was used to examine differences amongst pea cultivars. All analyses were performed at POS Bio-Sciences using a pico-tag amino acid analysis system (Waters Corporation, Milford, MA, USA) and high performance liquid chromatography (HPLC). In general, 15 amino acid residues were quantified according to the method developed by Bidlingmeyer et al. (1987), which involves adding 15 mL of 6 N HCl to ~20 mg of samples, and holding at 110ºC for 20 h to hydrolyze the proteins into individual amino acids prior to HPLC separation. The amount of sulfur-containing amino acids was determined according to Official Method 985.28 of AOAC International (AOAC, 2005) with some modifications, in which the addition of 1-octanol was omitted; 10 mL of cold performic acid was added to oxidize cysteine and methionine overnight at 4ºC, prior to protein hydrolysis with 15 mL of 6 N HCl at 110ºC for 16 h. The quantity of tryptophan was determined according to Official Method 988.15 of AOAC International (AOAC,

2005) with modifications, in which samples were hydrolyzed by treating with 10 M NaOH in a boiling water bath for 20 min, and then in an oven at 110ºC for 16 h prior to HPLC separation. All analyses were performed in duplicate.

3.7 Determination of legumin/vicilin ratio

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to characterize pea flours and pea protein isolates under non-reducing conditions. For both flours and isolates, 0.5 mL of 65.8 mM Tris-HCl buffer (2x Laemmli Sample Buffer, Bio-Rad Laboratories, Hercules, CA, USA) was added to a sample containing 1 mg of protein. Samples were vortexed (SP® Vortex Mixer, Baxter, Deerfield, IL, USA) on maximum speed for 30 s, heated at 95°C for 10 min (IncuBlock Model 285, Denville Scientific Inc., South Plainfield, NJ, USA) and then allowed to cool for 10 min at room temperature before centrifuging (Eppendorf Microcentrifuge Model 5424, Mississauga, ON) at 10,000 x *g* for 5 min. Five microlitres of each sample, for a final protein concentration of 10 µg per well, were loaded onto 4-20% gradient gels (Mini-PROTEON® TGX™ gel, Bio-Rad Laboratories) and fitted into a Mini-PROTEON® Tetra Cell (Bio-Rad Laboratories). Molecular mass markers of 10, 15, 25, 35, 40, 55, 70, 100, 130, and 170 kDa were also applied to a separate well (PageRuler Prestained Protein Ladder, Thermo Fisher Scientific Inc., Waltham, MA, USA). Samples were run for approximately 35 min at a constant 200V (PowerPAC™ HC Power Supply, Bio-Rad Laboratories) in Tris-glycine (pH 8.3) running buffer, stained for 30 min using 0.1% (w/v) Coomassie brilliant blue r-250 in 3:1:6 (v/v/v) methanol:acetic acid:water, and then destained overnight with three changes of the same solution without Coomassie brilliant blue. Destained gels were scanned (Epson Perfection V750 PRO scanner, Long Beach, CA, USA), and the resultant images were used to estimate the molecular masses of protein bands as well as to quantify the amount of protein present in each band using ImageQuant® (Ver. #8.1; Amersham Pharmacia Biotech., Piscataway, NJ, USA). Quantities of protein in bands were measured by volume, defined as the "sum of the pixel intensity for all pixels in a given selection" (Amersham Pharmacia Biotech.).

3.8 Surface characteristics of pea protein isolates

Protein solutions for both surface charge and hydrophobicity measurements were prepared by dispersing protein isolates (weight determined by protein content of isolates and amount of

protein required for each test) in 10 mM sodium phosphate buffer and adjusting to pH 7.0 using 0.1 M HCl or NaOH. All solutions were stirred for 1 h at room temperature, and used within 3 h of preparation. All tests were performed in triplicate unless noted otherwise.

3.8.1 Surface charge (zeta potential)

Overall surface charge for each protein isolate was determined according to Can Karaca et al. (2011). The electrophoretic mobilities (U_E) of 0.05% (w/w) protein solutions were measured at pH 7.0 using a Zetasizer Nano-ZS90 (Malvern Instruments, Westborough, MA, USA), at 25°C. Zeta potential (ζ) was calculated by applying U_E to Henry's equation:

$$U_E = \frac{2\varepsilon\zeta f(\kappa\alpha)}{3\eta} \quad \text{(eq. 3)}$$

where ε is permittivity, f(κα) is a function related to the ratio of particle radius (α) and Debye length (κ), and η is the dispersion viscosity. A Smoluchowski approximation f(κα) of 1.5 was assumed for this study, as is convention when using a folded capillary cell, and with samples of particles sizes larger than 0.2 μm dispersed in a moderately electrolytic solution (>1 mM). The Smoluchowski approximation assumes that: a) the concentration of particles (proteins) is sufficiently high such that the thickness of the electric double layer (Debye length) is small relative to the particle size (κα>>1); and b) ζ is linearly related to U_E (Somasundaran, 2006). All measurements were reported as the mean ± one standard deviation (n = 3).

3.8.2 Surface hydrophobicity (intrinsic fluorescence)

Intrinsic fluorescence of tryptophan was determined using a FluoroMax-4 spectrophotometer (Horiba Jobin Yvon Inc., Edison, NJ, USA) according to the method of Lam and Nickerson (2014). Protein solutions (0.05%, w/w) were passed through a 0.2-μm nylon syringe filter (VWR International, Mississauga, ON, Canada) to remove larger particles. Intrinsic fluorescence was detected at a constant excitation wavelength of 295 nm with a slit width of 2.5 nm, as a function of emission wavelength between 285 and 450 nm with a slit width of 5.0 nm, in 0.5 nm increments. A control spectrum of 10 mM sodium phosphate buffer without protein was subtracted from each protein solution spectrum, and the maximum fluorescence intensity (in

arbitrary units) was determined from the corrected spectra. All measurements were arbitrarily scaled down by a factor of 10,000, and reported as the mean ± one standard deviation (n = 3) using this value.

3.9 Functional properties of pea protein isolates

Protein solutions for all functional property measurements were prepared as described in Section 3.8.

3.9.1 Nitrogen solubility

Nitrogen solubility was measured using a modification of the method of Morr et al. (1985). Twenty gram of protein solution (1.0%, w/w), adjusted to pH 7.0, was centrifuged (VWR Clinical 200 centrifuge, VWR International) at 4180 x g for 10 min at room temperature. The nitrogen content of the supernatant was determined using a micro-Kjeldahl digestion and distillation unit (Labconco Corp., Kansas City, MO, USA). Solubility was calculated by dividing the nitrogen content in the supernatant sample by the nitrogen content of the isolate sample and expressing the result as a percentage. Nitrogen levels of isolates were re-measured using micro-Kjeldahl, because the protein analyzer used in Section 3.5.1 is not intended for wet samples. All measurements were reported as the mean ± one standard deviation (n = 3).

3.9.2 Oil holding capacity

Oil holding capacity (OHC) was determined according to a modification of the method of Ahmedna et al. (1999). Protein samples (0.5 g of protein) were dispersed in 5 g of canola oil in 50-mL centrifuge tubes, and vortexed (VWR Vortex Mixer, VWR International) for 10 s every 5 min on maximum speed, until 30 min had elapsed. Samples were centrifuged (VWR Clinical 200 centrifuge, VWR International) at 2000 x g for 10 min, followed by decanting of the supernatant. OHC was calculated as follows:

$$OHC\ (g/g) = \frac{(wet\ sample\ weight - dry\ sample\ weight)}{dry\ sample\ weight} \quad \text{(eq. 4)}$$

All measurements were reported as the mean ± one standard deviation (n = 3).

3.9.3 Foaming capacity and foam stability

Foaming capacity (FC) was measured using a modification of the method of Liu et al. (2010). In brief, 15 mL of a 1.0% (w/w) protein solution was foamed (T-10 Basic ULTRA-TURRAX®, IKA® Works, Inc., Wilmington, NC, USA) in a narrow glass beaker for 5 min at 8000 rpm using an S-10N – 10 g dispersing element positioned 1.7 mm below the air-water interface. Foamed solutions were then immediately transferred to a 50-mL graduated cylinder. FC was calculated as follows:

$$FC\ (\%) = \frac{foam\ volume}{initial\ sample\ volume} \times 100\% \quad \text{(eq. 5)}$$

After 30 min, the foam volume remaining from the FC test was measured, and foam stability (FS) was calculated as follows:

$$FS\ (\%) = \frac{foam\ volume\ after\ 30\ min}{initial\ foam\ volume} \times 100\% \quad \text{(eq. 6)}$$

All measurements were reported as the mean ± one standard deviation (n = 3).

3.9.4 Emulsion stability

Emulsion stability (ES) was determined according to Stone and Nickerson (2012) with minor modifications. In brief, 5 g of a 1.0% (w/w) protein solution and 5 g of canola oil were added to a 50-mL centrifuge tube and homogenized for 5 min at 8,000 rpm using a T-10 Basic ULTRA-TURRAX® homogenizer fitted with a S-10N – 10 g dispersing element, positioned 1.7 mm below the oil-water interface (IKA® Works, Inc.). The emulsion was immediately transferred to a 10-mL graduated cylinder (inner diameter = 10.5 mm; height = 160 mm, as measured by a digital caliper), where the volume of the aqueous layer separated after 30 min was noted. ES was calculated as follows, where V_B and V_A refer to the aqueous phase prior to homogenization (5 mL) and after 30 min of storage, respectively:

$$ES\ (\%) = \frac{V_B - V_A}{V_B} \times 100\%$$

(eq. 7)

All measurements were reported as the mean ± one standard deviation (n = 3).

3.10 Colour analysis

The colour of pea protein isolates was determined by colorimetry using a spectrophotometer (HunterLab MiniScan XE 45/0-L, Hunter Association Laboratory Inc., Reston, VA, USA) standardized using black and white tiles, according to the method of Withana-Gamage et al. (2011). Results were reported in units of L*, a*, and b* for brightness, redness, and yellowness, respectively. All measurements were reported as the mean ± one standard deviation (n = 3).

3.11 Statistical analysis

All statistical analyses were performed using SAS® 9.4 (SAS Institute Inc., Cary, NC, USA). SAS PROC MIXED was used to determine differences amongst means in the physicochemical and functional properties as a result of cultivar and environment, where cultivar was considered a fixed term while environment, field replicate, replicate (of laboratory tests), and cultivar × environment interactions were considered random terms. Where cultivar × environment interactions were significant, the dataset was separated according to the four environments (Saskatoon 2011, Saskatoon 2012, Rosthern 2011, Rosthern 2012) and mixed model analyses were independently performed for each environment (fixed term: cultivar; random terms: field replicate, replicate). Furthermore, datasets were also separated by location while combining year information (Saskatoon, Rosthern; fixed term: cultivar; random terms: year, year x cultivar interaction, field replicate, replicate), as well as sorted by year and merging location information (2011, 2012; fixed term: cultivar; random terms: location, location x cultivar interaction, field replicate, replicate), to allow investigation of the specific environmental factors impacting functionality means. Where cultivar × environment interactions were not significant, the dataset was analyzed as a whole. The Tukey test was employed as a post hoc procedure to differentiate differences amongst cultivars.

Prior to mixed model analysis, location, year, field replicate, and replicate data were assessed for homogeneity of variance using Levene's test. Variance for homogeneity of variance

in field replicate and laboratory replicate data were confirmed to be not significant ($p > 0.05$) before proceeding with mixed model analyses. Correlations between Lg/Vn ratio, surface properties, functional properties, and compositional analysis were examined using the Pearson product-moment correlation coefficient by the PROC CORR procedure. PROC GLM was employed to compare the functional characteristics of pea protein isolates against commercial protein isolate ingredients.

For all tests, significant differences were inferred at the 95% confidence level.

4. RESULTS AND DISCUSSION

4.1 Composition of pea flours and protein isolates

4.1.1 Thousand seed weight

Measurements of 1000-seed weight provide an indication of seed size variation, and are useful to producers for calculating seeding rates in targeting optimal crop densities (Anonymous, 2011a). The 1000-seed weights of the six pea cultivars in this study, which were grown in four environments, ranged from 167-275 g (Table 4.1). Overall, larger seeds were found for Cooper (mean = 258 g) and CDC Striker (mean = 235 g), whereas CDC Dakota (mean = 196 g) and CDC Golden (mean = 199 g) seeds were smaller. This is in agreement with data obtained through provincial testing in Saskatchewan, which found 1000-seed weights of 270 g and 205 g for Cooper and CDC Dakota, respectively (Anonymous, 2015).

Table 4.1. Weight (g) of 1000 seeds of six pea cultivars grown in four environments (two locations x two years). Superscripts within a column denote significant differences between cultivars ($p < 0.05$). Data represent the mean ± one standard deviation ($n = 2$).

Cultivar	Weight (g)			
	Saskatoon		Rosthern	
	2011	2012	2011	2012
Agassiz	229.9 ± 11.7^{b}	206.7 ± 4.0^{bc}	240.6 ± 2.1^{bc}	178.5 ± 12.6^{b}
CDC Dakota	206.3 ± 2.0^{b}	195.4 ± 0.8^{c}	201.8 ± 2.3^{e}	179.2 ± 3.5^{b}
CDC Golden	213.7 ± 5.5^{b}	201.2 ± 0.4^{bc}	213.3 ± 8.1^{de}	167.0 ± 6.4^{b}
CDC Striker	220.4 ± 1.6^{b}	233.1 ± 3.8^{a}	244.6 ± 4.3^{b}	239.9 ± 19.3^{a}
CDC Tetris	213.1 ± 6.6^{b}	211.9 ± 0.4^{b}	224.5 ± 2.1^{cd}	188.2 ± 3.3^{b}
Cooper	275.1 ± 4.0^{a}	244.8 ± 4.9^{a}	273.1 ± 3.9^{a}	237.1 ± 16.5^{a}

In addition, seed weights varied by environment, where seed grown in 2011 was larger than when grown in 2012 irrespective of location, although a comparison of overall 1000-seed weights for seed from Saskatoon and Rosthern did not reveal significant differences. When sorted by either year or location, the trend amongst cultivars persisted, where Cooper and CDC Striker seeds were larger, and CDC Dakota and CDC Golden seeds were smaller. Therefore, seed weight differences amongst samples were attributed largely to cultivar and to year of production, rather than to location.

Uniform seed weights are desired for establishing crops with similar rates of development (Anonymous, 2011a). The pea cultivars used in this study possessed 1000-seed weights generally comparable to those reported for these cultivars in provincial testing, but lacked uniformity across years; across cultivars, differences between 2011 and 2012 1000-seed weights ranged from 27 g (CDC Dakota) to 62 g (Agassiz). However, environmental differences due to year might have been mitigated in provincial testing data due to the use of much larger sample sizes (>20 field experiments tested in triplicate), which would give the appearance of greater uniformity year to year (Anonymous, 2015).

4.1.2 Proximate composition of flours and isolates

While differences in the protein content of peas grown in various environments have been reported previously (Acikgoz et al., 2009; Nikolopoulou et al., 2007; Wang et al., 2010), no significant cultivar × environment effects were found for the protein, ash, or lipid content of defatted pea flours or protein isolates in this study ($p > 0.05$). Pea flours exhibited protein contents in the range of 23.5-26.7% (d.b.) when separated by cultivar (Table 4.2). This is comparable to the results of Wang et al. (2010) who reported values ranging from ~21-28% for six pea cultivars. In this study, Cooper was found to have a significantly lower protein content than all other cultivars, except for Agassiz ($p < 0.05$), whereas ash (~3%, d.b.) and crude fat (0.3%, d.b.) contents were similar across cultivars ($p > 0.05$). Despite there being statistically significant differences in ash content amongst cultivars, a difference of ~0.2% was not deemed to be of practical significance. Black et al. (1998) also reported a small range in ash content across four pea types as sorted by seed coat appearance (3.2-3.3%, d.b.), whereas Can Karaca et al. (2011) obtained a higher crude fat content (1%, d.b.) for defatted pea flour relative to the results of the present study.

Table 4.2. Mean protein, ash, and crude fat levels in defatted pea flours as a function of cultivar, and reported on a dry basis (d.b.). Values for protein and ash contents were derived from all samples (n = 16), whereas crude fat contents were derived from one field replicate (n = 8). Superscripts within a column denote significant differences between cultivars ($p < 0.05$). Data represent the mean ± one standard deviation (n = 16).

Cultivar	Protein (%, d.b.)	Ash (%, d.b.)	Crude fat (%, d.b.)
Agassiz	25.2 ± 2.2^{ab}	3.1 ± 0.1^{ab}	0.3 ± 0.0^{a}
CDC Dakota	26.0 ± 1.3^{a}	3.0 ± 0.1^{bc}	0.3 ± 0.1^{a}
CDC Golden	25.4 ± 2.0^{a}	3.2 ± 0.2^{a}	0.3 ± 0.0^{a}
CDC Striker	26.7 ± 1.5^{a}	3.0 ± 0.2^{c}	0.3 ± 0.0^{a}
CDC Tetris	25.4 ± 1.9^{a}	3.0 ± 0.1^{c}	0.3 ± 0.1^{a}
Cooper	23.5 ± 1.5^{b}	3.1 ± 0.2^{ab}	0.3 ± 0.0^{a}

High protein contents, in the range of 89.7-92.5% (d.b.), were achieved in the pea protein isolates (Table 4.3). Reflecting the results for pea flour, the protein isolate prepared from Cooper had the lowest protein content, which was significantly lower than the protein contents of the isolates from CDC Striker and CDC Tetris ($p < 0.05$). Differences amongst cultivars were also found for ash (6.2-7.3%, d.b.) and total lipid (2.3-3.5%, d.b.) content. Cooper exhibited a higher ash content than all other cultivars, except for CDC Golden. Meanwhile, for total lipid content (the sum of crude fat and polar lipid), the highest levels were measured in CDC Tetris and Cooper, whereas the lowest was in CDC Dakota. The pea protein isolates had protein contents similar to those reported by Shevkani et al. (2015) for five pea genotypes (~91-95%, d.b.), but the values were slightly higher than those obtained by Toews and Wang (2013) (~86-87%, d.b.) for two cultivars; both authors reported lower levels of ash (3.6-5.7%, d.b.). Following a similar extraction procedure as used in the current study, Can Karaca et al. (2011) found that the ash content of a pea protein isolate was 5.9% (d.b.) vs. 2.9% (d.b.) in the original flour.

Table 4.3. Mean protein, ash, and total lipid levels in pea protein isolates as a function of cultivar, and reported on a dry basis (d.b.). Values for protein and ash contents were derived from all samples (n = 16), whereas total lipid contents were derived from one field replicate (n = 8). Superscripts within a column denote significant differences between cultivars ($p < 0.05$). Data represent the mean ± one standard deviation (n = 16).

Cultivar	Protein (%, d.b.)	Ash (%, d.b.)	Total lipid[1] (%, d.b.)
Agassiz	90.9 ± 1.6^{ab}	6.4 ± 1.0^{b}	2.5 ± 0.2^{c}
CDC Dakota	91.0 ± 1.6^{ab}	6.3 ± 0.3^{b}	2.3 ± 0.1^{d}
CDC Golden	91.1 ± 1.5^{ab}	6.8 ± 0.5^{ab}	2.7 ± 0.1^{c}
CDC Striker	92.5 ± 1.3^{a}	6.4 ± 0.4^{b}	2.8 ± 0.1^{b}
CDC Tetris	91.7 ± 1.5^{a}	6.2 ± 0.4^{b}	3.5 ± 0.2^{a}
Cooper	89.7 ± 1.7^{b}	7.3 ± 1.2^{a}	3.4 ± 0.0^{a}

[1] Sum of crude fat and polar lipid

4.1.3 Extraction yield

The amount of protein isolate recoverable from flour was reported as isolate yield, whereas the percent of total protein present in flour which was recovered in the isolate was termed protein yield. No significant cultivar × environment interactions were found for either parameter ($p > 0.05$). Isolate yield, when sorted by cultivar, ranged from ~17-19% (d.b.); a higher isolate yield was obtained for CDC Striker and CDC Dakota compared to Cooper ($p < 0.05$) (Table 4.4). Meanwhile, protein yield was found to range from ~70-74% (d.b.), with no difference amongst cultivars ($p > 0.05$). Using a similar extraction method as employed in this study for three pea cultivars, Stone et al. (2015b) reported slightly lower isolate yields (~15-16%, d.b.) and a wider range for protein yield (~63-77%, d.b.), with no differences between cultivars. Overall, the extraction method employed in this study was able to achieve consistent extraction yields across all samples.

Table 4.4. Isolate yield and protein yield of pea protein isolates as a function of cultivar, reported on a dry basis (d.b.). Superscripts within a column denote significant differences between cultivars ($p < 0.05$). Data represent the mean ± one standard deviation (n = 8).

Cultivar	Isolate yield[1] (%, d.b.)	Protein yield[2] (%, d.b.)
Agassiz	18.7 ± 1.5^{ab}	73.5 ± 1.4^{a}
CDC Dakota	19.1 ± 0.9^{a}	72.5 ± 1.7^{a}
CDC Golden	18.0 ± 1.3^{ab}	70.3 ± 1.5^{a}
CDC Striker	19.2 ± 1.2^{a}	72.6 ± 2.6^{a}
CDC Tetris	18.0 ± 1.2^{ab}	70.3 ± 2.4^{a}
Cooper	17.2 ± 1.1^{b}	71.3 ± 3.1^{a}

[1] Amount of protein isolate material produced from flour

[2] Amount of protein in flour retained in extracted isolate

4.1.4 Amino acid composition of flours and isolates

The amino acid composition (mol %) was similar across all cultivars and between pea flours and pea protein isolates, with no difference greater than ±1% found for any amino acid (Tables 4.5 and 4.6). However, it should be noted that results in this section were derived from only one field replicate in one environment, and therefore might be less reliable than results of other analyses in this study. Overall, samples were most abundant in glutamic acid (and glutamine) and aspartic acid (and asparagine), and were also high in arginine, lysine, and leucine. Methionine, cysteine, and tryptophan were found in lowest concentrations in the pea samples. These results are in agreement with literature values for the most prominent and scarce amino acids in pea globulins (Jackson et al, 1969; Sikorski, 2011).

In a comparison of changes in amino acid composition from pea flour to protein isolate, the greatest increases were found in arginine (mean = 0.9%, SD = 0.1) and leucine (mean = 0.7%, SD = 0.1), and the greatest decreases were found in aspartic acid (mean = -0.7%, SD = 0.2) and threonine (mean = -0.6%, SD = 0.2) (Table 4.7). Although the changes were small, the decreases observed across all six cultivars for some amino acids indicate that the alkaline

Table 4.5. Amino acid composition of pea flours derived from six cultivars. Seed samples were from one field replicate grown at Rosthern, SK in 2011. Values were normalized as the percentage of total amino acids detected represented by each amino acid (n = 2).

Amino acid (mol %)	**Agassiz**	**CDC Dakota**	**CDC Golden**	**CDC Striker**	**CDC Tetris**	**Cooper**
Non-essential amino acids						
Aspartic acid (+ Asparagine)	12.8	12.1	12.1	12.4	12.2	12.4
Glutamic acid (+ Glutamine)	17.2	17.2	17.4	17.6	17.0	17.0
Serine	5.6	5.4	5.4	5.4	5.3	5.4
Glycine	4.3	4.4	4.5	4.2	4.3	4.4
Arginine	8.2	8.8	8.7	9.0	9.1	8.6
Alanine	4.5	4.5	4.5	4.3	4.4	4.5
Proline	4.6	4.6	4.7	4.5	4.6	4.6
Tyrosine	3.4	3.8	3.5	3.2	3.7	3.7
Cysteine	1.4	1.4	1.6	1.3	1.4	1.6
Essential amino acids						
Histidine	3.1	3.4	3.4	3.3	3.2	3.2
Threonine	4.1	4.2	4.3	4.2	4.2	4.2
Valine	4.5	4.6	4.5	4.4	4.4	4.5
Methionine	0.9	1.0	1.0	0.9	1.0	1.0
Isoleucine	4.2	4.1	3.9	4.0	4.0	4.2
Leucine	7.6	7.4	7.3	7.5	7.4	7.5
Phenylalanine	5.2	5.0	4.9	4.9	4.8	5.0
Lysine	7.4	6.9	7.3	7.8	7.9	7.4
Tryptophan	1.0	1.1	1.1	1.1	1.1	1.1

Table 4.6. Amino acid composition of pea protein isolates derived from six cultivars. Seed samples were from one field replicate grown at Rosthern, SK in 2011. Values were normalized as the percentage of total amino acids detected represented by each amino acid (n = 2).

Amino acid (mol %)	Agassiz	CDC Dakota	CDC Golden	CDC Striker	CDC Tetris	Cooper
Non-essential amino acids						
Aspartic acid (+ Asparagine)	11.9	11.6	11.6	11.6	11.5	11.6
Glutamic acid (+ Glutamine)	17.6	17.4	17.1	17.8	17.5	17.5
Serine	5.6	5.4	5.4	5.4	5.4	5.5
Glycine	3.8	3.9	3.9	3.8	3.9	3.9
Arginine	9.2	9.7	9.6	9.9	9.9	9.5
Alanine	3.9	4.1	4.1	3.8	3.9	4.1
Proline	4.5	4.6	4.6	4.7	4.8	4.6
Tyrosine	4.1	4.2	4.3	4.1	4.2	4.1
Cysteine	0.8	1.0	1.1	0.9	1.0	0.9
Essential amino acids						
Histidine	2.8	3.0	2.9	2.9	2.8	2.8
Threonine	3.8	3.6	3.8	3.6	3.4	3.6
Valine	4.5	4.5	4.5	4.5	4.5	4.5
Methionine	0.8	0.9	1.0	0.8	0.9	0.8
Isoleucine	4.3	4.2	4.1	4.2	4.2	4.2
Leucine	8.2	8.1	8.0	8.3	8.2	8.2
Phenylalanine	5.4	5.3	5.2	5.2	5.2	5.3
Lysine	7.9	7.6	7.6	7.6	7.7	7.8
Tryptophan	0.9	1.0	1.1	0.9	1.0	1.0

Table 4.7. Change in amino acid composition between pea flours and pea protein isolates. Seed samples were from one field replicate grown at Rosthern, SK in 2011.

Amino acid (mol %)	Agassiz	CDC Dakota	CDC Golden	CDC Striker	CDC Tetris	Cooper
Non-essential amino acids						
Aspartic acid (+ Asparagine)	-0.9	-0.5	-0.5	-0.8	-0.7	-0.8
Glutamic acid (+ Glutamine)	0.4	0.2	-0.3	0.2	0.5	0.5
Serine	0.0	0.0	0.0	0.0	0.1	0.1
Glycine	-0.5	-0.5	-0.6	-0.4	-0.4	-0.5
Arginine	1.0	0.9	0.9	0.9	0.8	0.9
Alanine	-0.6	-0.4	-0.4	-0.5	-0.5	-0.4
Proline	-0.1	0.0	-0.1	0.2	0.2	0.0
Tyrosine	0.7	0.4	0.8	0.9	0.5	0.4
Cysteine	-0.6	-0.4	-0.5	-0.4	-0.4	-0.7
Essential amino acids						
Histidine	-0.3	-0.4	-0.5	-0.4	-0.4	-0.4
Threonine	-0.3	-0.6	-0.5	-0.6	-0.8	-0.6
Valine	0.0	-0.1	0.0	0.1	0.1	0.0
Methionine	-0.1	-0.1	0.0	-0.1	-0.1	-0.2
Isoleucine	0.1	0.1	0.2	0.2	0.2	0.0
Leucine	0.6	0.7	0.7	0.8	0.8	0.7
Phenylalanine	0.2	0.3	0.3	0.3	0.4	0.3
Lysine	0.5	0.7	0.3	-0.2	-0.2	0.4
Tryptophan	-0.1	-0.1	0.0	-0.2	-0.1	-0.1

extraction/isoelectric precipitation process was more successful in recovering globulins (pI ~ pH 4.4-4.6) over albumins (pI ~ pH 6.0) (Swanson, 1990). Of the nutritionally-essential amino acids, albumins are more abundant in lysine, threonine, tryptophan, and methionine, as compared to globulins (Boye et al., 2010b; Damodaran et al., 2008). All of these amino acids decreased in every cultivar except for some inconsistencies in lysine, which is also abundant in the vicilin proteins (Schroeder, 1982). Rubio et al. (2013) reported the lysine content of vicilin (~111 g/kg dry matter) to be more than twice that of legumin (~50 g/kg). However, despite differences in Lg/Vn ratio amongst pea cultivars, differences in amino acid composition were not reflected in their profiles. One possible explanation is in the mechanism of quantification of amino acids versus that employed for legumin and vicilin proteins. Proteins must be dissociated into certain expected forms in order to convey the molecular masses that were included in the Lg/Vn ratio estimate; whereas for amino acid analysis, proteins were hydrolyzed into individual amino acids prior to detection, thus protein conformation would not affect results. Schroeder (1982) reported that sulfur amino acid content did not vary between pea cultivars with high and low levels of legumin, and concluded that protein content, rather than cultivar, affects amino acid content.

4.1.5 Ratio of legumin and vicilin proteins

Under non-reducing condition in SDS-PAGE, pea flours and protein isolates separated into bands having molecular masses of ~10 kDa to ~105 kDa. The hexameric legumin proteins were found to have dissociated into monomers (α+β fractions) with an apparent molecular mass of ~60 kDa (Figures 4.1 and 4.2) (Tzitzikas et al., 2006). Because no protein had accumulated in the gel loading zone, it was assumed that all of the legumin proteins had dissociated into smaller monomers and therefore were able to enter the gel. Non-covalent bonds maintaining the hexameric structure of legumin were broken by SDS and heat, whereas the disulfide bond between α and β residues remained intact without the addition of a reducing agent such as β-mercaptoethanol (Barac et al., 2010). The trimeric vicilin proteins appeared as both intact and dissociated monomeric fractions, with bands detected at ~50 kDa (α+β+γ fractions), ~30-37 kDa (α+β fractions), and ~14-20 kDa (α, β, and γ fractions) (Tzitzikas et al., 2006). The band at ~70 kDa corresponded to the molecular mass of convicilin as reported by O'Kane et al. (2004). The cluster of bands at ~100 kDa were assumed to be lipoxygenase, which generally exhibit molecular masses of 94-104 kDa in plants (Szymanowska et al., 2009). Several other protein bands were visible in the SDS-PAGE

profiles of pea flours, but were not found in the pea protein isolates, with the most apparent and recurrent such band located at ~80 kDa. However, these bands were not believed to be either legumin or vicilin, and therefore were not included in the Lg/Vn ratio calculations.

For pea flour, the Lg/Vn ratio means ranged from 0.38 to 0.81, and from 0.36 to 0.79 for pea protein isolates. This is in agreement with multiple sources cited in the literature, with the exception of one author who reported Lg/Vn ratios upward of 8.0 (Barac et al., 2010; Gueguen & Barbot, 1988; Mertens et al., 2012; Schroeder, 1982). The interaction of cultivar × environment was not significant in the analysis of Lg/Vn ratio means for pea flours or protein isolates ($p > 0.05$). Therefore, results across all environments were studied as a whole as a function of cultivar. The lack of a cultivar × environment interaction was also reported by Casey et al. (1982), Nikolopoulou et al. (2007), and Gueguen & Barbot (1988), but this contradicts the findings of Bourgeois et al. (2009) and Mertens et al. (2012). In particular, Bourgeois et al. (2009) reported a significant increase in the Lg/Vn ratio when pea was grown in extreme conditions of high heat and low rainfall. According to Nikolopoulou et al. (2007), low rainfall is related to increased protein content in pea, and positive correlations between protein content and Lg/Vn ratio have been observed by other authors (Cousin et al., 1992; Gueguen & Barbot, 1988; Swanson, 1990). In this study, the Lg/Vn ratio of pea protein isolates was not found to correlate significantly with protein content ($p > 0.05$). One possible explanation is that although samples were grown in environments having different rainfall accumulations (201.9 to 439.2 mm), sufficient rainfall was received to avert significant moisture-related environmental stress (Table 3.2). Paired with similarly moderate temperatures across all environments (Table 3.3), Lg/Vn ratios and protein contents likely were reflective of intrinsic differences amongst cultivars rather than differences due to environmental conditions.

For pea flours, the order of cultivars with respect to Lg/Vn ratio, from highest to lowest numerical mean, was CDC Golden, CDC Dakota, CDC Striker, CDC Tetris, Cooper, and Agassiz (Table 4.8). The ratio means for CDC Golden and CDC Dakota were similar, whereas the ratio for CDC Golden was higher than that of the other four cultivars ($p < 0.01$). The ratio mean for CDC Dakota was similar to that of CDC Striker, and that of CDC Tetris was comparable to those of both CDC Striker and Cooper. The lowest Lg/Vn ratio mean was observed for Agassiz, which was significantly different from those of the other cultivars ($p < 0.001$).

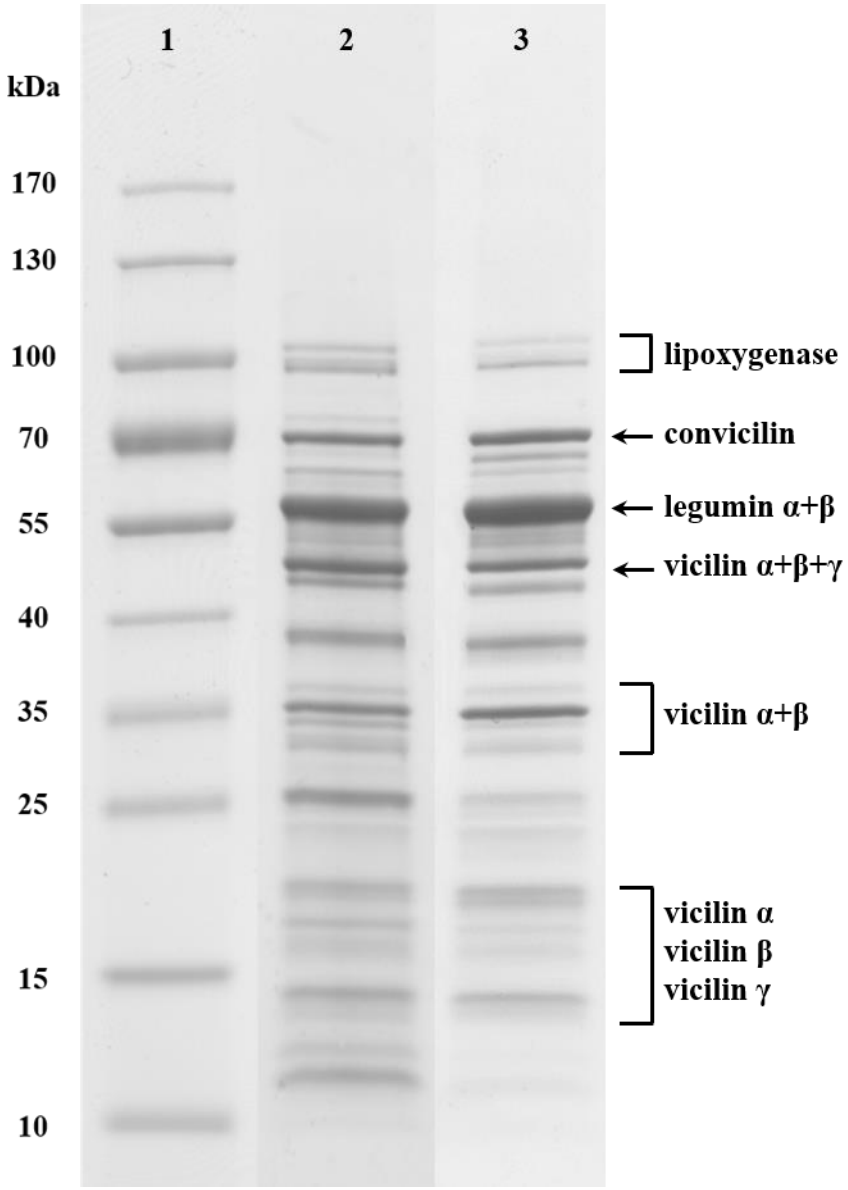

Figure 4.1. Example of non-reducing SDS-PAGE of pea. Lanes: (1) molecular weight marker, (2) pea flour (CDC Dakota, Saskatoon, SK, 2011), and (3) pea protein isolate (CDC Golden, Rosthern, SK, 2012).

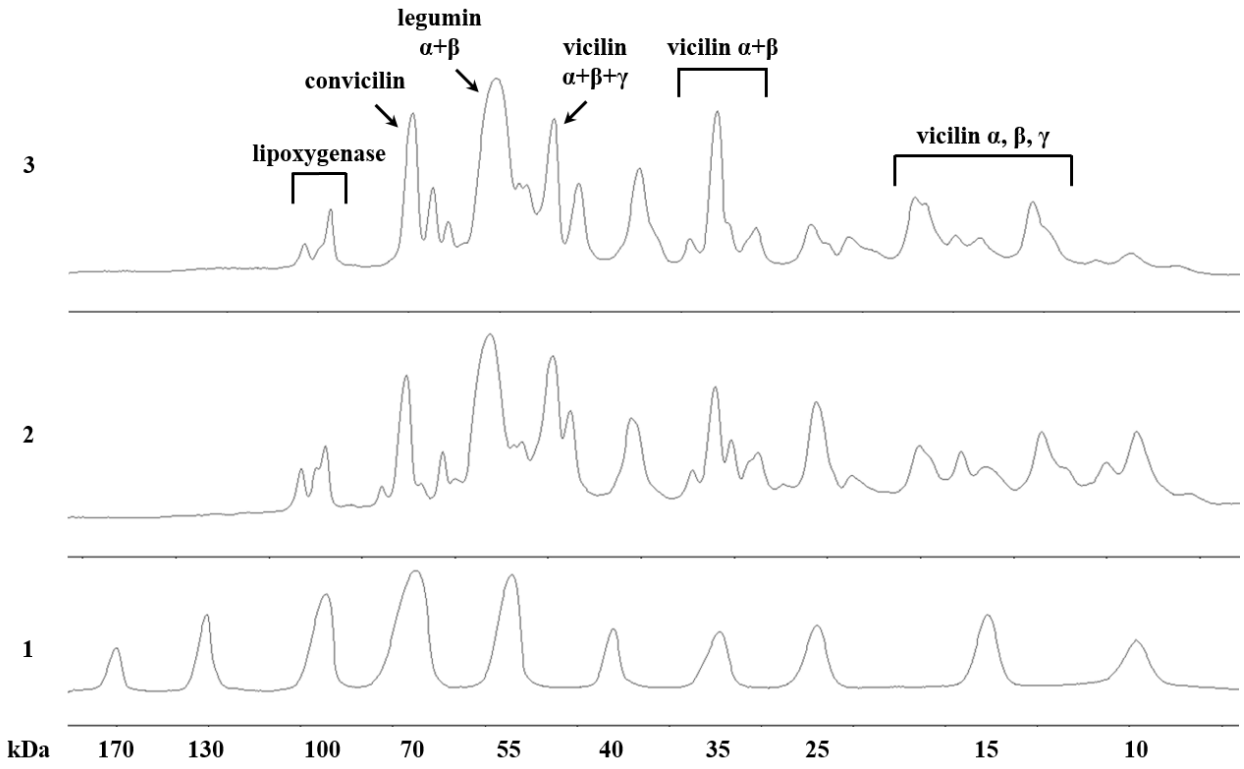

Figure 4.2. Densitometry analysis profile corresponding to the SDS-PAGE shown in Figure 4.1. Lanes (1) molecular weight marker, (2) pea flour (CDC Dakota, Saskatoon, SK, 2011), and (3) pea protein isolate (CDC Golden, Rosthern, SK, 2012).

The same order of ranking of Lg/Vn ratio means by cultivar was observed for the pea protein isolates, and comparisons of ratio means were similar. CDC Golden and CDC Dakota had significantly higher ratio means compared to the other cultivars ($p < 0.001$). The ratio means for CDC Striker and CDC Tetris were similar, but higher than those for Cooper and Agassiz ($p < 0.05$). As was the case for pea flours, Agassiz exhibited the lowest Lg/Vn ratio mean ($p < 0.001$) amongst pea protein isolates.

The Lg/Vn ratios of the pea protein isolates were slightly lower (1-12% decrease) than those of the corresponding pea flours (Table 4.8). This indicates that the alkaline extraction/isoelectric precipitation process did not favour the recovery of legumin vs. vicilin or vice versa, at least not substantially. The conservation of the Lg/Vn ratio from flour to pea protein isolates may have been assisted by the close physical proximity of legumin and vicilin proteins in pea, as both are located in the same protein bodies (Gueguen, 1983; Shewry et al., 1995). However,

the net decrease in Lg/Vn ratio observed across all cultivars, albeit small enough perhaps to be deemed negligible, suggests that recovery of vicilin vs. legumin might have been slightly favoured using this protein isolation method.

Table 4.8. Lg/Vn ratios of pea flours and pea protein isolates as a function of cultivar, and net change in Lg/Vn ratio means from flour to protein isolates. Superscripts within a column denote significant differences between cultivars ($p < 0.05$). Data represent the mean ± one standard deviation ($n = 24$).

Cultivar	Legumin/Vicilin ratio		
	Pea flours	Pea protein isolates	Net change (flour → isolate)
Agassiz	0.38 ± 0.06^{e}	0.36 ± 0.05^{d}	-0.02
CDC Dakota	0.78 ± 0.15^{ab}	0.77 ± 0.10^{a}	-0.01
CDC Golden	0.81 ± 0.14^{a}	0.79 ± 0.13^{a}	-0.02
CDC Striker	0.71 ± 0.12^{bc}	0.63 ± 0.12^{b}	-0.08
CDC Tetris	0.64 ± 0.11^{cd}	0.62 ± 0.08^{b}	-0.02
Cooper	0.60 ± 0.11^{d}	0.52 ± 0.08^{c}	-0.08

Despite differences in the Lg/Vn ratio of pea protein isolates by cultivar, the Lg/Vn ratio was only significantly and weakly correlated to two functional attributes – solubility ($r = -0.21$; $p < 0.01$) and ES ($r = -0.19$; $p < 0.05$), yet a moderately strong correlation existed between Lg/Vn ratio and surface hydrophobicity (H_0) ($r = 0.62$; $p < 0.001$) (Table 4.9). The Lg/Vn ratio is a measure of protein composition, hence the value obtained is independent of the surface and functional characteristics of the isolate. Meanwhile, results of functional tests are dependent on H_0. In other words, H_0 might change markedly when measured under conditions that influence the degree of protein unfolding, which would lead to differences in functional properties. However, the Lg/Vn ratio would remain unchanged. Accordingly, it is logical for relationships between the Lg/Vn ratio and functional properties to be few and weak.

Correlation patterns suggest that given the right processing conditions, the implications of differences in Lg/Vn ratio amongst pea protein isolates are minimal. Structure-function relationships in pea protein isolates will be further discussed in subsequent sections.

Table 4.9 Pearson product-moment correlation coefficients between the Lg/Vn ratios of pea protein isolates, surface characteristics, functional properties, and composition (protein, ash, lipid). Correlations of significance ($p < 0.05$) are indicated (*).

	Lg/Vn	H_0	ZP	Sol	OHC	FC	FS	ES	Protein	Ash	Lipid
Lg/Vn	1.000	0.6247*	-0.1516	-0.2081*	0.0427	-0.0482	0.0414	-0.1916*	0.1297	-0.0235	-0.1385
p		<.001	0.070	0.012	0.612	0.566	0.622	0.021	0.208	0.821	0.348
H_0		1.000	-0.0493	0.3170*	0.2342*	0.1654*	0.1763*	-0.1053	0.4065*	-0.0431	-0.2074
p			0.557	<.001	0.005	0.048	0.035	0.209	<.001	0.677	0.157
ZP			1.000	0.0252	-0.0178	0.0219	0.0470	0.0352	0.1283	-0.1587	0.2478
p				0.764	0.832	0.794	0.576	0.676	0.213	0.123	0.090
Sol				1.000	0.2784*	0.3683*	0.2763*	0.0851	0.3553*	-0.0362	0.1483
p					<.001	<.001	<.001	0.311	<.001	0.726	0.315
OHC					1.000	-0.0914	-0.0479	-0.0293	0.3756*	0.0127	-0.0831
p						0.276	0.568	0.728	<.001	0.902	0.574
FC						1.000	0.6261*	0.0690	0.1807	-0.1455	0.0187
p							<.001	0.412	0.078	0.157	0.900
FS							1.000	0.0463	0.1834	-0.1428	-0.0268
p								0.582	0.074	0.165	0.857
ES								1.000	-0.1429	0.0718	0.4813*
p									0.165	0.487	<.001
Protein									1.000	-0.4015*	-0.2783*
p										<.001	.055
Ash										1.000	0.3616*
p											0.012
Lipid											1.000
p											

Abbreviations: Lg/Vn, legumin/vicilin ratio of pea protein isolates; H_0, surface hydrophobicity; ZP, zeta potential; Sol, solubility; OHC, oil holding capacity; FC, foaming capacity; FS, foaming stability; ES, emulsion stability

4.2 Surface characteristics of pea protein isolates

4.2.1 Surface charge (zeta potential)

Zeta potential (ZP) is a measure of the electrical potential across a plane of shear, located between the stern and diffuse layers surrounding a dispersed particle (Anonymous, 2011b). Zeta potential is affected by the ionic strength (or conductivity) and pH of the solvent, which determines the diffuse layer thickness and the degree of ionization of amino acid residues (Clogston & Patri, 2011; Malhotra & Coupland, 2004). Because ZP values were acquired by measuring

electrophoretic mobility, conditions that impact particle velocity, such as the viscosity or dielectric constant of the medium, would also affect ZP (Anonymous, 2011b).

Overall, ZP measurements were in the range of -23.0 mV to -24.2 mV. Due to the lack of a cultivar × environment interaction, ZP data was analyzed for differences amongst cultivars regardless of environmental effects (year, location). No significant differences in ZP means were found amongst cultivars ($p > 0.05$) (Table 4.10). Also, no significant correlations were found between ZP and any other physicochemical or functional properties ($p > 0.05$) (Table 4.9).

Table 4.10. Surface charge (zeta potential) (mV) of pea protein isolates as a function of cultivar at pH 7.0. Superscripts within a column denote significant differences between cultivars ($p < 0.05$). Data represent the mean ± one standard deviation ($n = 24$).

Cultivar	Zeta potential (mV)
Agassiz	-23.6 ± 1.1[a]
CDC Dakota	-24.3 ± 0.8[a]
CDC Golden	-23.6 ± 1.3[a]
CDC Striker	-23.7 ± 1.3[a]
CDC Tetris	-23.0 ± 1.6[a]
Cooper	-23.7 ± 1.0[a]

The solvent pH of 7.0 used was above the pea globulin pI of ~4.5, resulting in a net negative charge in all samples. Surface charge is dominated by the sum of the amino acids in greatest abundance on the protein surface (Malhotra & Coupland, 2004). Due to very similar amino acid compositions across the six pea cultivars, similar configurations due to protein folding and the degree of amino acid ionization were hypothesized to be present within all samples when tested under identical solvent conditions. The results of this study were comparable to values reported in the literature for multiple legume sources extracted using a number of different methods. Can Karaca et al. (2011) reported ZP means in the range of -18.3 mV to -23.0 mV for protein isolates prepared at laboratory-scale from pea, soy, chickpea, faba bean, and lentil by alkaline extraction/isoelectric precipitation or salt extraction. Similarly, Stone et al. (2015b) tested isolates

prepared at laboratory-scale from three pea cultivars (one from each of three market classes – green, yellow, and dun) using alkaline extraction/isoelectric precipitation, salt extraction, and micellar precipitation, and reported ZP means of -20.5 mV to -21.6 mV. Accordingly, it can be concluded that the nature of the solvent is the primary factor affecting ZP, irrespective of protein source and extraction method.

A high ZP (less than -30 mV or more than +30 mV) indicates stable proteins in solution due to strong electrostatic repulsion between proteins, whereas a low ZP (between -30 mV and +30 mV) suggests weaker repulsive forces and a greater tendency toward protein-protein aggregation (Anonymous, 2011b). Low ZP results obtained from the present study suggest that pea protein isolates are unstable in solution and will tend toward aggregation at pH 7.0.

4.2.2 Surface hydrophobicity

The surface hydrophobicity (H_0) of a protein can be attributed to its conformation (shape and size), amino acid composition and sequence, and intra- and intermolecular interactions (Wang et al., 2014). In particular, the hydrophobic interactions from hydrophobic amino acids and hydrophobic side chains on polar amino acids are integral to the final folded structure of proteins (Arunachalam & Gautham, 2008; Moelbert et al., 2004). Although nonpolar groups tend to orient towards the protein core, a number of hydrophobic patches present on the protein surface are believed to contribute to the functional properties of a protein (Hall, 1996).

In the literature, H_0 has frequently been detected using fluorescent probe binding methods, such as with the 8-anilino-1-naphthalenesulfonic acid (ANS) probe for aromatic residues, or the *cis*-parinaric acid (CPA) probe for aliphatic residues (Moro et al., 2001). However, H_0 results obtained using such methods are comparable to those from intrinsic fluorescence detection (Moro et al., 2001; Lam & Nickerson, 2014).

Surface hydrophobicity was measured as the maximum intrinsic fluorescence emitted by tryptophan for each of the pea protein isolates. Although other aromatic amino acids – phenylalanine and tyrosine – also exhibit intrinsic fluorescence, their emissions have been excluded due to the inconsistencies of low detection and quenching, respectively (Wrolstad et al., 2004). Overall, H_0 means were found to range between 195 and 267 a.u. (arbitrary units) depending on the cultivar and environment (year and location). Analysis of cultivar means was performed separately for each environment, due to a significant interaction between cultivar and environment

(Table 4.11). Pea protein isolates produced from seed grown at Rosthern in 2012 were found to be the most hydrophobic (mean = 242 a.u.), whereas isolates prepared from seed grown at Saskatoon in 2012 were the least hydrophobic (mean = 214 a.u.).

Table 4.11. Maximum intrinsic fluorescence (arbitrary units, a.u.) of pea protein isolates prepared from seed of six pea cultivars grown in four environments (two locations x two years), at pH 7.0. Superscripts within a column denote significant differences between cultivars ($p < 0.05$). Data represent the mean ± one standard deviation ($n = 6$).

Cultivar	Maximum intrinsic fluorescence (a.u.)			
	Saskatoon		Rosthern	
	2011	2012	2011	2012
Agassiz	204 ± 13^{b}	195 ± 7^{b}	216 ± 4^{b}	224 ± 12^{d}
CDC Dakota	229 ± 8^{ab}	232 ± 7^{a}	228 ± 10^{ab}	252 ± 12^{ab}
CDC Golden	233 ± 26^{a}	227 ± 17^{a}	234 ± 5^{a}	267 ± 8^{a}
CDC Striker	234 ± 33^{a}	213 ± 5^{ab}	231 ± 17^{ab}	237 ± 5^{bcd}
CDC Tetris	234 ± 7^{a}	222 ± 21^{a}	229 ± 10^{ab}	227 ± 10^{cd}
Cooper	219 ± 8^{ab}	197 ± 7^{b}	231 ± 7^{ab}	243 ± 12^{bc}

Across all four environments, no differences in H_0 means were found between CDC Golden and CDC Dakota ($p > 0.05$), which yielded the highest H_0 values. H_0 means ranged from 227 a.u. (Saskatoon 2012) to 267 a.u. (Rosthern 2012) for CDC Golden, and from 228 a.u. (Rosthern 2011) to 252 a.u. (Rosthern 2012) for CDC Dakota. Agassiz, for which means ranged from 195 a.u (Saskatoon 2012) to 224 a.u. (Rosthern 2012), consistently displayed the lowest H_0, and was the only cultivar different from CDC Golden in all environments ($p < 0.05$). CDC Striker, Cooper, and CDC Tetris generally exhibited H_0 values in the middle ranges with no identifiable pattern, having the same means as either CDC Golden or Agassiz, or both, depending on the environment.

When the data was sorted by location (with no year x cultivar interaction) or by year (with no location x cultivar interaction), similar trends were observed for isolates prepared from seed grown at Saskatoon or in 2011, where no differences in H_0 were found between CDC Golden and

CDC Dakota, but both exhibited differences from Agassiz. Mean values for H_0 for CDC Golden, CDC Dakota, and Agassiz, respectively, were 230 a.u., 231 a.u., and 199 a.u. for isolates prepared from seed grown at Saskatoon, and 233 a.u., 229 a.u., and 210 a.u. for isolates prepared from seed grown in 2011.

Although no differences in H_0 due to cultivar were found for isolates prepared from seed grown at Rosthern or in 2012, the occurrence of significant differences in H_0 due to cultivar of isolates prepared from seed grown at Rosthern in 2012 indicates that both location and year contributed to the differences in H_0 observed amongst cultivars.

Surface hydrophobicity measurements can indicate the degree of protein unfolding in solution and changes in the distribution of surface hydrophobic patches (Moro et al., 2001; Wang et al., 2014). Possible implications for functional performance will be explored in Section 4.3. In this study, H_0 was found to be positively correlated with Lg/Vn ratio in a moderately strong manner ($r = 0.62$, $p < 0.001$) (Table 4.9). This is most apparent for CDC Golden and CDC Dakota, with the greatest H_0 and Lg/Vn ratios, and for Agassiz which exhibited the lowest H_0 and Lg/Vn ratio. One possible explanation for the relationship is that legumin not only comprises more tryptophan than vicilin, but as a larger protein with more surface area, also allows greater exposure of tryptophan (Koyoro & Powers, 1987). Another possible explanation is that vicilin is intrinsically a more hydrophilic protein than legumin due to its N-terminal extension composed mainly of serine, glutamic acid, and aspartic acid, which are polar amino acid residues (Liang & Tang, 2013; Sikorski, 2001).

Using the ANS probe binding method to compare the H_0 of protein isolates prepared from four genotypes of kidney bean, which comprise mainly vicilin, against those of field pea, Shevkani et al. (2015) reported markedly lower H_0 in kidney bean (mean = 269 a.u.) than in field pea (mean = 563 a.u.). The authors also reported significant H_0 differences amongst genotypes. Similar conclusions were drawn by Koyoro and Powers (1987) who used the CPA probe binding method and found legumin to be more hydrophobic (11.2 a.u.) than vicilin (2.2 a.u.) when purified from a globulin mixture with a H_0 of 7.6 a.u. Similarly, Liang and Tang (2013) found the H_0 value of pea legumin to be almost two-fold that of vicilin, using the ANS probe binding method.

4.3 Functional properties of pea protein isolates

4.3.1 Solubility

The mean solubility of pea protein isolates ranged from 62.5-75.2%. Due to a significant interaction between cultivar and environment, analysis of cultivar means was performed separately for each environment (Table 4.12). Overall, the solubility of protein isolates produced from pea grown in Rosthern 2012 (mean = 71.6%) was higher than that of pea grown in other environments (mean range = 67.6-67.8%). Differences were found amongst cultivars grown in all environments except for Saskatoon 2012.

Table 4.12. Solubility (%) of pea protein isolates prepared from seed of six pea cultivars grown in four environments (two locations x two years), at pH 7.0. Superscripts within a column denote significant differences between cultivars ($p < 0.05$). Data represent the mean ± one standard deviation (n = 6).

Cultivar	Solubility (%)			
	Saskatoon		Rosthern	
	2011	2012	2011	2012
Agassiz	69.0 ± 0.6^{a}	69.0 ± 3.8^{a}	70.4 ± 0.6^{a}	75.2 ± 3.3^{a}
CDC Dakota	66.2 ± 3.0^{ab}	67.0 ± 1.4^{a}	62.5 ± 1.6^{c}	68.0 ± 1.4^{c}
CDC Golden	64.1 ± 2.3^{b}	67.5 ± 1.1^{a}	66.5 ± 0.8^{b}	72.0 ± 2.6^{ab}
CDC Striker	69.6 ± 4.3^{a}	68.1 ± 1.3^{a}	67.7 ± 3.3^{ab}	73.2 ± 2.1^{a}
CDC Tetris	70.1 ± 2.2^{a}	68.0 ± 2.9^{a}	70.2 ± 3.7^{ab}	69.2 ± 2.5^{bc}
Cooper	66.4 ± 1.2^{ab}	66.9 ± 2.8^{a}	69.2 ± 1.3^{ab}	72.1 ± 2.4^{ab}

Across the four environments, protein isolates from Agassiz, CDC Striker, and Cooper displayed similarly high solubility. CDC Dakota and CDC Golden generally had the lowest solubility, showing significant differences from Agassiz in two of four environments each (Rosthern 2011 and Rosthern 2012 for CDC Dakota; Saskatoon 2011 and Rosthern 2011 for CDC Golden) ($p < 0.05$). Specifically, the solubility of CDC Dakota protein isolates prepared from pea grown at Rosthern in 2011 was significantly lower than that of all other cultivars, whereas for Rosthern 2012 it was similar only to that of CDC Tetris. Aside from Rosthern 2012, protein isolates from CDC Tetris yielded amongst the highest solubility in all other environments. CDC Golden

was not uniquely lowest in solubility in any environment, being similar to both CDC Dakota and Cooper for Saskatoon 2011, and to all cultivars except Agassiz and CDC Dakota for Rosthern 2011. The importance of cultivar to the solubility of pea protein isolates is supported by sources in the literature who also utilized the alkaline extraction/isoelectric precipitation method. Barac et al. (2010) reported solubility means of 70-87% for six pea genotypes, whereas Shevkani et al. (2015) found means to range from 64-80% for five pea genotypes. Both authors observed significant differences between every genotype tested. Stone et al. (2015a) also found significant differences amongst seven pea cultivars tested, with solubility means of 54-76%.

When sorted by location (without a year x cultivar interaction), no differences were detected in solubility means between cultivars from Saskatoon (mean range = 65.8-69.0%) or Rosthern (mean range = 65.3-72.8%). Likewise, cultivars displayed similar solubility means in both 2011 (mean range = 64.4-70.1%) and 2012 (mean range = 67.5-71.9%) when sorted by year (without a location x cultivar interaction). The appearance of significant differences between cultivar means upon combining location and year to form environments suggests some form of interaction between factors related to location and year, although the influence of such factors is difficult to isolate and requires further study. Environmental factors might be successful in masking differences caused intrinsically by cultivars, as suggested by the minimal difference between cultivar means in Saskatoon 2012 (2%) compared to the other three environments (6-8%).

Protein solubility is dependent on the proportion and distribution of hydrophilic and hydrophobic groups on the surface of a protein molecule, which in turn is dictated by a number of intrinsic (amino acid composition and distribution, molecular flexibility, charge, isoelectric point) and extrinsic (solution pH, temperature, ionic strength) factors (Hall, 1996; Stone et al., 2015a). Because all pea protein isolate samples were subjected to the same extrinsic conditions during solubility testing, differences detected can be attributed to intrinsic factors.

Significant correlations were found between solubility and most other parameters tested, although the relationships were usually weak (Table 4.9). Solubility was weakly correlated to Lg/Vn ratio ($r = -0.21$, $p < 0.01$). The negative relationship can be ascribed to three sources. Firstly, vicilin is likely a more hydrophilic protein than legumin due to its end terminal extension; therefore an increase in solubility can be expected when the proportion of vicilin in solution is increased. Koyoro and Powers (1987) determined vicilin to have much higher solubility (96.9%) compared to legumin (73.6%) at pH 7.0, whereas Liang and Tang (2013) found both proteins to be similar

in solubility (legumin = 78%; vicilin = 80%). Such discrepancies are reflected in the weak correlation between solubility and Lg/Vn ratio. Secondly, Kimura et al. (2008) observed that under neutral and weakly alkaline conditions, N-glycosylation of carbohydrate moieties enhanced protein solubility significantly. N-glycosylation is common in vicilin proteins, but has not been found in legumin, and therefore supports the higher solubility of vicilin over legumin (Swanson, 1990). Thirdly, in terms of amino acid composition, the negatively charged glutamic acid and aspartic acid are able to bind water more tightly than water is able to bind to itself, and therefore contributes more to solubility than other amino acid residues (Kramer et al., 2012). Vicilin has been reported to comprise either similar or greater levels of glutamic acid and aspartic acid than legumin (Koyoro & Powers, 1987; Rubio et al, 2013). However, the highly heterogeneous nature of vicilin proteins might also explain the weak relationship between solubility and Lg/Vn ratio (Sikorski, 2001).

While only a moderately weak correlation was found between solubility and H_0 ($r = 0.32$, $p < 0.001$), it is stronger than that between solubility and Lg/Vn ratio. Although higher H_0 would intuitively correlate to decreased solubility, the opposite relationship was found to be the case. Authors who came across a similar finding proposed that as H_0 increased to a certain degree, hydrophobic proteins precipitated and decreased the overall solubilized protein content. By the H_0 measurement methods employed, only hydrophobic fractions remaining in solution could be detected. Accordingly, protein solubility was observed to decrease despite decreasing H_0 (Shevkani et al., 2015; Wagner et al., 2000). From the perspective of this study, because the correlation was moderately weak, tryptophan residues on protein surfaces were only partially responsible for precipitation.

Surprisingly, no significant correlation was detected between solubility and total lipid content ($p > 0.05$). Because lipid residues are small relative to proteins, they might be embedded primarily in the protein core upon interaction with hydrophobic proteins, and therefore did not affect the protein surface where interaction with the solvent occurs (Alzagtat & Alli, 2002). Meanwhile, solubility was found to be correlated to protein content in a positive, moderately weak manner ($r = 0.36$, $p < 0.001$), despite sample solutions were prepared to consist of the same protein concentration. An explanation for the relationship is that since protein content is negatively correlated to both ash and total lipid content ($r = -0.40$, $p < 0.001$; $r = -0.28$, $p < 0.05$, respectively),

samples of more highly purified protein isolates contained less insoluble contaminants of ash and total lipid, and experienced less hindrance to solubility.

Aside from emulsion stability, moderately weak but significant positive relationships were found between solubility and all other functional properties tested. Solubility has been deemed crucial to the functionality of proteins, and these relationships will be further explored in subsequent sections (Damodaran et al., 2008).

4.3.2 Oil holding capacity

Oil holding capacity (OHC) is defined as the amount of oil that can be absorbed per gram of protein (Lin & Zayas, 1987). An interaction effect was not found between cultivar and environment ($p > 0.05$), therefore all OHC data was analyzed as a function of cultivar irrespective of environment. Overall, OHC means were ranged from 3.1-3.3 g/g, with no significant differences between cultivars (Table 4.13).

Table 4.13. Oil holding capacity (g oil/g protein) of pea protein isolates as a function of cultivar. Superscripts within a column denote significant differences between cultivars ($p < 0.05$). Data represent the mean ± one standard deviation (n = 24).

Cultivar	Oil holding capacity (g/g)
Agassiz	3.3 ± 0.7^{a}
CDC Dakota	3.2 ± 0.5^{a}
CDC Golden	3.2 ± 0.5^{a}
CDC Striker	3.1 ± 0.6^{a}
CDC Tetris	3.3 ± 0.4^{a}
Cooper	3.1 ± 0.5^{a}

Similar results were obtained by Stone et al. (2015b), who also found no difference between OHC means of three pea cultivars (3.5-3.8 g/g). Although no cultivar differences were observed by Stone et al. (2015a) amongst seven pea cultivars, the authors reported lower OHC means (1.1-1.4 g/g). Likewise, Boye et al. (2010a) reported a lower OHC value compared to the

results of this study for one pea cultivar (~1.2 g/g), whereas Withama-Gamage et al. (2011) found a similar value (~2.9 g/g).Although the mechanism of OHC is not fully understood, it is conceived to be the physical entrapment of oil with influences from the hydrophobicity and amino acid distribution of proteins (Chau & Cheung, 1998). The OHC test employed in this study involved the dispersion of proteins in canola oil. Because hydrophobic bonds are largely responsible for the interaction between proteins and fatty acids, H_0 was expected to positively affect OHC (Alzagtat & Alli, 2002). In this study, OHC was weakly but positively correlated to both H_0 ($r = 0.23$, $p < 0.01$) and solubility ($r = 0.28$, $p < 0.001$) (Table 4.9). Intuitively, however, OHC and H_0 should be inversely related to solubility, which suggests that the relationship discussed in Section 4.3.1 applies here. As described, as H_0 increased, the hydrophobic proteins precipitated and resulted in overall decreased solubility, but with a greater proportion of hydrophilic proteins remaining in solution. However, as only solubilized proteins can be detected by the H_0 technique, H_0 measurements were observed to decrease as well. Accordingly, the positive correlation between OHC and solubility was likely driven indirectly by the positive influence of H_0 on OHC.

While legumin was presumed to comprise more tryptophan and therefore to possess a higher H_0 compared to vicilin, no significant correlation was observed between OHC and the Lg/Vn ratio ($p > 0.05$). It is possible that the effects of Lg/Vn ratio were negated by conformational changes in proteins incurred in the hydrophobic environment of canola oil, as well as by the physical changes required to entrap oil to form protein-lipid complexes. After testing protein isolates from four genotypes of each of kidney bean and pea, of which kidney bean proteins have much greater vicilin contents than do pea proteins, Shevkani et al. (2015) reported that OHC means for pea (mean = 6.4 g/g; range = 5.5-7.2 g/g) were only slightly higher than those of kidney bean (mean = 5.9 g/g; range = 4.7-6.9 g/g). The study conducted by Shevkani et al. (2015) supports the observation that neither legumin nor vicilin proteins demonstrated any particular influence on OHC.

Finally, a moderately weak, positive relationship was observed between protein content and OHC ($r = 0.38$, $p < 0.001$). The protein contents of samples were taken into account when conducting the OHC test in this study. However, because protein content was found to be negatively correlated with total lipid content ($r = -0.28$, $p < 0.05$), it is reasonable that in samples where fewer protein-lipid complexes were formed with lipids naturally occurring in pea protein

isolates, a greater amount of free protein would be available to interact and adsorb to the surrounding canola oil, thus increasing OHC.

4.3.3 Foaming capacity

In this study, mean values for the foaming capacity (FC) of pea protein isolates ranged from 167.4-243.7%. Such values are generally comparable to those in the literature for pea protein isolates prepared by isoelectric precipitation. For example, Stone et al. (2015b) reported FC means ranging from 155.0-183.0% for three cultivars. However, Barac et al. (2010) measured FCs as high as 325% for one of six pea genotypes tested (mean range = 175-325%), whereas a lower value (102%) was obtained for one cultivar by Boye et al. (2010a).

Analysis of cultivar means was performed separately for each environment due to a significant cultivar × environment interaction (Table 4.14). Of the four environments, pea protein isolates produced from pea grown in Saskatoon in 2011 (mean = 216.3%; mean range = 185.7-243.7%) and Rosthern in 2012 (mean = 216.3%; mean range = 195.8-235.0%) yielded the highest FCs. Meanwhile, the lowest FCs were found in pea protein isolates produced from peas grown in Saskatoon in 2012 (mean = 194.3%; mean range = 177.3-233.2%) and Rosthern in 2011 (mean = 193.3%; mean range = 167.4-219.0%).

Table 4.14. Foaming capacities (%) of pea protein isolates prepared from seed of six pea cultivars grown in four environments (two locations x two years), at pH 7.0. Superscripts within a column denote significant differences between cultivars ($p < 0.05$). Data represent the mean ± one standard deviation (n = 6).

Cultivar	Foaming capacity (%)			
	Saskatoon		Rosthern	
	2011	2012	2011	2012
Agassiz	243.7 ± 22.6^{a}	189.6 ± 28.9^{ab}	219.0 ± 11.7^{a}	195.8 ± 13.0^{b}
CDC Dakota	206.0 ± 24.2^{ab}	196.6 ± 24.4^{ab}	167.4 ± 16.2^{c}	208.1 ± 13.2^{ab}
CDC Golden	209.7 ± 27.6^{ab}	233.2 ± 12.3^{a}	188.3 ± 16.8^{bc}	218.2 ± 5.3^{ab}
CDC Striker	215.2 ± 26.0^{ab}	177.3 ± 31.0^{b}	189.7 ± 16.4^{abc}	235.0 ± 20.3^{a}
CDC Tetris	237.2 ± 16.5^{a}	183.2 ± 11.6^{ab}	206.8 ± 16.0^{ab}	211.8 ± 24.9^{ab}
Cooper	185.7 ± 21.6^{b}	186.0 ± 15.4^{ab}	188.3 ± 24.0^{bc}	228.8 ± 15.8^{a}

For Saskatoon 2011, Agassiz and CDC Tetris had higher FC than did Cooper. For Saskatoon 2012, CDC Golden was found to have greater FC than did CDC Striker. Meanwhile, CDC Striker and Cooper exhibited greater FC compared to Agassiz for Rosthern 2012 pea protein isolates. For Rosthern 2011, the FC of Agassiz was higher than that of CDC Dakota, CDC Golden, and Cooper, of which CDC Dakota exhibited amongst the lowest FC but was similar to all cultivars except for Agassiz and CDC Tetris.

When data was analyzed by FC means between cultivars as a function of location (with no year x cultivar interaction), no differences were found within either Saskatoon (mean range = 185.8-217.8%) or Rosthern (mean range = 187.8-212.3%) ($p > 0.05$). Likewise, FC means as a function of year (with no location x cultivar interaction) also resulted in no differences between cultivars for seed grown in 2012 (mean range = 192.7-221.9%) ($p > 0.05$). However, seed grown in 2011 yielded protein isolates with significantly higher FC for the cultivar Agassiz (mean = 231.3%) compared to Cooper (mean = 187.0%) and CDC Dakota (mean = 186.7%). Because neither Saskatoon nor Rosthern nor the year 2012 alone showed differences in FC between cultivars, it is believed that a combination of factors conferred by both location and year was the cause of differences found for Saskatoon 2012 and Rosthern 2012. However, the exact interaction between location and year, and the contribution of either, requires further study. Meanwhile, since differences in FC means were found between cultivars in 2011, environmental factors within the year 2011 were assumed to be the main cause of differences between cultivars for the Saskatoon 2011 and Rosthern 2011 environments.

For all other properties tested which exhibited significant cultivar × environment interactions (i.e., H_0, solubility), certain behaviours displayed by a cultivar, such as the low H_0 of Agassiz, persisted in a similar manner throughout most or all environments. However, no obvious pattern of either high or low FC could be found for any of the six cultivars. Accordingly, it is likely that intrinsic properties of different pea cultivars did not contribute to FC as strongly as extrinsic factors such as environment.

The lack of or a low contribution of intrinsic factors to FC was supported by the absence of a significant correlation between FC and Lg/Vn ratio ($p > 0.05$). Foam formation requires new air cells to be formed and stabilized at a faster rate than their collapse (Kinsella, 1981). Traditionally, high FC is believed to be a result of quick adsorption of protein molecules to the air/water interface and the flexibility of proteins to rearrange to an optimal conformation at the

interface, which is assisted by the more hydrophobic surfaces of partially denatured proteins (Damodaran, 2005; Damodaran et al., 2008). Accordingly, it was expected that a lower Lg/Vn ratio would correlate to a higher FC. Legumin is a more rigid protein containing disulfide bonds, whereas vicilin, with a smaller, more flexible structure due to a lack of disulfide bonds, should have greater capacity to migrate and re-orient at the air/water interface (Dagorn-Scaviner et al., 1986). However, such a relationship was not observed in this study. Shevkani et al., (2015) tested protein isolates from four genotypes each of pea and kidney bean, and found that despite the much higher vicilin component in kidney bean isolates compared to pea, both materials exhibited similar FCs (mean range = 83-121% for kidney bean isolates and 87-132% for pea protein isolates).

Wierenga and Gruppen (2010) explained a more recent model of foam formation, where proteins that migrate near the air/water interface selectively adsorb, and that the conformation of proteins in solution undergoes negligible change upon adsorption to the interface. Accordingly, FC would not be governed by the rate of protein migration or flexibility, but rather by its affinity to the air/water interface. Results of this study show some support for this idea, where FC is significantly correlated to solubility ($r = 0.37$, $p < 0.001$) and H_0 ($r = 0.17$, $p < 0.05$), although it is important to note that correlations are moderately weak and weak, respectively (Table 4.9). Higher solubility increases the availability of proteins migrating toward the interface and therefore the likelihood of adsorption, whereas greater H_0 increases the affinity of a protein for the interface. In fact, Wierenga and Gruppen (2010) stated that a minimum H_0 must be exhibited by proteins as a criterion for air/water interface adsoption. In a study performed by Koyoro and Powers (1987), the authors found that foam formation by sparging was not possible at pH 7.0 using legumin, vicilin, or a mixture of both proteins. However, heating the protein solutions prior to sparging made foam formation possible by partially denaturing proteins and greatly increasing H_0.

Aside from solubility, H_0, and FS, significant correlations were not observed between FC and any other property tested. Total lipid content was expected to deter FC, as lipid is favoured over protein at the interface due to its higher surface activity, yet it lacks the cohesiveness and viscoelasticity to withstand the pressure of foam formation (Damodaran et al., 2008). Similar to how the expectation for lipid to restrict solubility also was not met, it is possible that lipid was mostly buried at the protein core where hydrophobic clusters mainly reside (Alzagtat & Alli, 2002).

4.3.4 Foam stability

Foam stability (FS) results did not indicate a cultivar × environment interaction, therefore results from all environments were analyzed together by cultivar (Table 4.15). Overall, FS means ranged from 73.5-75.2%, with no significant differences found between cultivars ($p > 0.05$). Slightly lower results were reported by Stone et al. (2015b), who also measured remaining foam volume after a 30-min wait time. They found FS means to range from 68.0-69.6% for protein isolates prepared from three cultivars of pea using alkaline extraction/isoelectric precipitation. Much lower FS results have been reported by other authors. Boye et al. (2010a) measured FS of 43% after 5 min for one pea cultivar. Meanwhile, Barac et al. (2010) measured FS of ~35% for two pea genotypes after only 3 min. However, much higher FS results were obtained by Shevkani et al. (2015) who found the mean range for four pea genotypes to be 94-96% after a 30-min wait time.

Table 4.15. Foam stability (%) of pea protein isolates as a function of cultivar at pH 7.0. Superscripts within a column denote significant differences between cultivars ($p < 0.05$). Data represent the mean ± one standard deviation ($n = 24$).

Cultivar	Foam stability (%)
Agassiz	74.9 ± 2.0^{a}
CDC Dakota	74.1 ± 2.2^{a}
CDC Golden	75.0 ± 2.6^{a}
CDC Striker	75.2 ± 2.2^{a}
CDC Tetris	75.3 ± 2.1^{a}
Cooper	73.5 ± 2.4^{a}

Foam stability refers to the ability of a protein to stabilize a foam against stresses and maintain its volume, and is traditionally governed by film cohesiveness and thickness as well as flexibility to deform against stresses (Damodaran et al., 2008; Hall, 1996). Similar to the reasoning for FC, since vicilin is a smaller and more flexible protein compared to legumin due to its lack of disulfide bonds, a negative correlation was expected between FS and Lg/Vn ratio. However, no

relationship was found, as was observed between FC and Lg/Vn ratio ($p > 0.05$). Against all properties tested, FS was significantly correlated only to FC ($r = 0.63$, $p < 0.001$), solubility ($r = 0.28$, $p < 0.001$), and H_0 ($r = 0.18$, $p < 0.05$) (Table 4.9). Foam stability is influenced seemingly by the same parameters as FC, which is supported by their moderately strong correlation to each other, as well as their mutually and exclusively weak positive correlations with solubility and H_0.

According to the view expressed by Wierenga and Gruppen (2010), foam formation is subject to protein availability and affinity for the air/water interface, which in turn are influenced by solubility and H_0, respectively. If FC and FS are governed by similar factors, the importance of protein availability and affinity for the air/water interface to FS pertains to how distances between adsorbed proteins affect protein-protein interactions at the interface (Wierenga & Gruppen, 2010). In order to produce a thick, cohesive film, low repulsion between adsorbed proteins is desirable to maximize protein-protein interactions. Because all samples were tested at pH 7.0 and therefore at the same magnitude from pI, repulsive forces between proteins should be reflected in ZP measurements. No differences in ZP were found between cultivars, and as expected, no differences in FS were found either. Surprisingly, no significant correlation was observed between ZP and FS. As discussed in Section 4.2.1, ZP is dictated by conditions of the sample solution, such as pH and ionic strength. Therefore, both FS and ZP might have been impacted by an extrinsic factor, such as pH, rather than affecting each other directly. Also, to further complicate matters, a balance of two antagonistic factors – high solubility and low repulsion – is needed to achieve sufficient FS.

Overall, it can be concluded that all foams stabilized by pea protein isolates in this study were unstable, due to a volume loss of ~25% after only 30 min.

4.3.5 Emulsion stability

The ability of a protein-stabilized emulsion to resist destabilization is referred to as emulsion stability (ES). In this study, ES means ranged from 95.1-96.1%. No significant interaction between cultivar and environment was observed ($p > 0.05$), hence combined data across all environments were analyzed for ES differences between cultivars (Table 4.16). ES was found to be significantly higher for the cultivar Cooper compared to CDC Golden ($p < 0.05$), whereas all other cultivars displayed ES means similar to each other as well as to both Cooper and CDC Golden.

Table 4.16. Emulsion stability (%) of pea protein isolates as a function of cultivar at pH 7.0. Superscripts within a column denote significant differences between cultivars ($p < 0.05$). Data represent the mean ± one standard deviation (n = 24).

Cultivar	Emulsion stability (%)
Agassiz	95.8 ± 1.4^{ab}
CDC Dakota	95.7 ± 1.2^{ab}
CDC Golden	95.1 ± 1.3^{b}
CDC Striker	95.7 ± 1.2^{ab}
CDC Tetris	96.0 ± 1.0^{ab}
Cooper	96.1 ± 0.9^{a}

Using the same method as in this study, Stone et al. (2015b) reported ES means of 96.7-99.9% for isolates prepared from three pea cultivars using alkaline extraction/isoelectric precipitation. Meanwhile, Koyoro and Powers (1987) found that legumin, vicilin, and mixed globulin fractions of pea exhibited similar ES (mean range = 56.6-59.0%) by measuring the water retained in an emulsified state after subjecting emulsions to centrifugal stress. Other authors tested ES using the emulsion stability index (ESI), which measures the ability of an emulsion to resist changes over time (Boye et al., 2010a). Whereas no differences in ESI were found by Stone et al. (2015a) amongst seven cultivars (mean range = 11.0-11.3 min), Barac et al. (2010) observed significant differences between seven genotypes (mean range = 25-80 min). Differences in ESI were also reported by Shevkani et al. (2015) for five pea genotypes (mean range = 52.6-95.4 min).

Emulsions are naturally prone to phase separation due to the increase in interfacial free energy, rendering them thermodynamically unstable (Waltra, 2003). Proteins work to decrease interfacial tension, by aligning at the interface where the hydrophobic train formation lies along the interface, and the hydrophilic tail and loop formations protrude outward into the aqueous phase creating steric hindrance between neighbouring droplets (Damodaran, 2005). The results indicated that a weak, negative correlation existed between ES and the Lg/Vn ratio ($r = -0.19$, $p < 0.05$) (Table 4.9). This inverse relationship was expected, because the more flexible structure of vicilin, due to a lack of disulfide bonds, should more readily conform to train, tail, and loop formation.

Although proteins possessing more rigid structures, such as legumin, can form thicker and more cohesive interfacial films with time, their capacity for stabilizing emulsions is compromised by their slower rate of adsorption and lower affinity for the interface, compared to other more surface active materials (Dagorn-Scaviner et al., 1987; Damodaran, 2005).

The weak nature of the correlation between ES and Lg/Vn ratio might be explained by the presence of small, amphiphilic surfactant molecules in the form of phospholipids. Total lipid, which comprises crude fat fractions and phospholipids, was positively and moderately correlated to ES ($r = 0.48$, $p < 0.001$). Small surfactant molecules, with their rapid diffusion and high surface activity, compete with and partially displace proteins at the O/W interface (Alzagtat & Alli, 2002). However, without the capability to re-orient and produce a cohesive film around the dispersed phase, small surfactant molecules are not as effective as larger proteins in stabilizing emulsions (Damodaran, 2005). The greater affinity of vicilin over legumin for the interface, coupled with displacement of proteins by phospholipids, may explain the weak negative correlation between ES and Lg/Vn ratio, and support why ES is largely unchanged amongst cultivars regardless of Lg/Vn ratio differences.

Aside from the Lg/Vn ratio and total lipid content, no significant correlation was found between ES and any other property tested. Zeta potential was expected to correlate positively to ES, as it is a measurement of repulsion between dispersed droplets. However, both ZP and ES might be primarily dependent on a third factor – pH – rather than having an influence on each other (Shevkani et al., 2015).

From the results of this study, a statistical difference was found between means of ES amongst cultivars. However, from a practical viewpoint, an overall difference in means of ~1% between Cooper and CDC Golden is negligible, and it can be concluded that no difference existed between the six cultivars for ES. Overall, a decrease in ES of ~4-5% was observed after a 30-min drainage period. Accordingly, it would be expected that further phase separation would occur with time.

4.4 Colour of pea protein isolates

Food ingredients that impart noticeable visual effects on a product can negatively impact consumer acceptability of the final product (Khouryieh & Aramouni, 2012; Toews & Wang, 2013). Significant differences were found between the combined means of yellow and green

cotyledon peas for all three parameters of brightness (L*), redness (a*), and yellowness (b*) ($p < 0.001$) (Table 4.17). Overall, yellow peas yielded higher measurements for all parameters, with the most pronounced difference in the degree of redness. Cultivars of the same cotyledon colour, namely yellow pea, also exhibited colour differences. CDC Dakota was found to be less red than other yellow peas, whereas its degree of yellowness was comparable to both Agassiz (yellow) and CDC Tetris (green). However, it displayed less brightness than other yellow cultivars as well as CDC Striker (green). Meanwhile, all three green pea cultivars exhibited similar brightness, redness, and yellowness.

Table 4.17. Colour of pea protein isolates as a function of cultivar and cotyledon colour.[1] Superscripts within a column denote significant differences between cultivars ($p < 0.05$). Data represent the mean ± one standard deviation (n = 24).

Cultivar	L*	a*	b*
Yellow cotyledon			
Agassiz	79.0 ± 1.9^{a}	12.0 ± 0.8^{a}	32.3 ± 1.6^{b}
CDC Dakota	72.9 ± 1.2^{d}	10.2 ± 0.4^{b}	30.9 ± 1.1^{bc}
CDC Golden	77.7 ± 1.7^{b}	13.1 ± 0.7^{a}	36.1 ± 1.5^{a}
Mean	*76.5 ± 3.1*	*11.7 ± 1.4*	*33.1 ± 2.6*
Green cotyledon			
CDC Striker	74.3 ± 1.3^{c}	5.5 ± 1.0^{c}	28.7 ± 1.0^{d}
CDC Tetris	73.6 ± 1.8^{cd}	4.5 ± 0.5^{c}	29.8 ± 1.2^{cd}
Cooper	73.9 ± 1.9^{cd}	5.3 ± 0.7^{c}	28.7 ± 1.2^{d}
Mean	*73.9 ± 1.7*	*5.1 ± 0.9*	*29.0 ± 1.2*

[1] L* = black (0) to white (100); a* = green (-) to red (+); b* = blue (-) to yellow (+)

In this study, all pea flours were defatted using hexane prior to protein extraction. The green and yellow colours in pea are primarily due to the presence of chlorophyll and carotenoid pigments, respectively, which would have been partially solubilized by hexane and removed during the defatting process (Damodaran et al, 2008; Edelenbos et al., 2001). In addition, Withana-Gamage et al. (2011) reported higher L* values in defatted chickpea protein isolates from loss of

oil-soluble pigments. In the commercial production of pea protein isolates, by which the fat removal process might be omitted, protein products could be expected to assume darker, more pronounced colours since less pigments are removed.

From the colour results for the six pea cultivars, green peas can be used interchangeably without imparting visual differences amongst cultivars, whereas some differences existed between yellow pea cultivars. In particular, CDC Dakota exhibited comparable colour characteristics to both yellow and green cultivars. Overall, yellow pea cultivars might be more desirable for procurement by product developers, since their lighter tinge should be easier to mask than green, thus having less negative impact on end product acceptability. Producers of commercial pea protein isolates should find it advantageous that cultivars of the same cotyledon colour can be dismissed as a relevant factor when sourcing seed to achieve protein blends. A much wider selection of pea becomes available to producers when consistent end product appearances can be achieved using a number of different pea cultivars as raw ingredients.

4.5 Comparison between pea protein isolates and commercial protein isolates

The functionality of six commercial protein isolates derived from pea (PPIc – Nutri-Pea Ltd., Portage la Prairie, MB), whey (WPI – Davisco Foods International, Inc., Le Sueure, MN), egg (EPI – Ballas Egg Products Corp., Zanesville, OH), wheat (WhPI – ADM, Decatur, IL), and soy (SPI 221 – Cargill Health & Food Technologies, Wayzata, MN; SPI 974 – ADM, Decatur, IL) were evaluated alongside the pea protein isolates (PPI) prepared at laboratory scale. The commercial isolates exhibited substantial differences in protein, ash, and total lipid (crude fat + polar lipid) content (Table 4.18). SPI 974 was found to have the highest protein level at ~95% (d.b.), followed by EPI, SPI 221, and PPI (~90%), and by WPI, PPIc, and WhPI at ~83%. Based on the protein levels, it appeared that all of the commercial ingredients, with the exception of WhPI, had been processed using a wet extraction method, likely under alkaline conditions, to select for the albumin- and globulin-type proteins. For WhPI, a water-washing process was likely employed to remove starch and solubles from gluten (Day et al., 2006).

The high ash content in SPI 221, EPI, and PPIc (6.1-8.5% d.b.) may suggest that the extraction medium contained higher NaCl levels to favour the extraction of globulin-type proteins over albumins. In addition, the extraction of 7S globulin proteins is favoured over the extraction of 11S proteins in dilute salt (Grant & Lawrence, 1964). Consequently, it is proposed that SPI 221,

due to its higher ash content, contained higher levels of glycinin than β-conglycinin, whereas a greater amount of β-conglycinin was present in SPI 974 due to its lower ash content. Depending on the proteins present, differences in functionality can be expected. Presumably, a low level of NaCl would have been used during WPI production to promote the solubility of both albumin and globulin proteins via a salting-in process, which promotes protein-water interactions by creating greater order within the hydration layers surrounding the protein. It is likely that all proteins were precipitated by adjusting the pH to the respective isoelectric point, with the exceptions of WhPI, where starch and solubles were removed from gluten using water, and WPI, which would have been ultrafiltered (Day et al., 2006; Yee et al., 2007).

Table 4.18. Protein, ash, and total lipid contents of six commercial protein isolates (n = 2) and pea protein isolates prepared at laboratory scale from six pea cultivars (protein, ash: n = 96; total lipid: n = 48). Superscripts within a column denote significant differences between materials ($p < 0.05$). Data represent the mean ± one standard deviation.

Material	Protein (%, d.b.)	Ash (%, d.b.)	Total lipid[1] (%, d.b.)
Soy, Prolisse ISE-221 (SPI 221)	89.5 ± 0.4^{b}	6.1 ± 0.1^{b}	0.7 ± 0.0^{d}
Soy, PRO-FAM 974® (SPI 974)	94.9 ± 0.1^{a}	3.3 ± 0.9^{c}	1.7 ± 0.1^{cd}
Whey (WPI)	83.5 ± 0.3^{c}	2.9 ± 0.0^{cd}	2.6 ± 0.0^{bc}
Pea (PPIc)	83.5 ± 0.1^{c}	8.5 ± 0.5^{a}	7.1 ± 0.1^{a}
Wheat (WhPI)	83.4 ± 0.3^{c}	0.6 ± 0.0^{d}	1.5 ± 0.0^{cd}
Egg (EPI)	88.8 ± 0.1^{b}	6.5 ± 0.1^{ab}	1.1 ± 0.0^{d}
Pea protein isolates (PPI)[2]	91.1 ± 1.7^{b}	6.6 ± 0.8^{ab}	2.9 ± 0.4^{b}

[1] Sum of crude fat and polar lipid

[2] Derived from all PPI values (protein, ash: n = 96; total lipid: n = 48)

The total lipid content comprises neutral and polar lipids. In all cases, with the exception of PPIc, it appears that a defatting process was employed prior to extraction. In particular, soy protein is processed from flakes derived from the oil extraction process (El-Shemy, 2011; Singh et al., 2008). The EPI may or may not have been defatted, as the fat content of egg white is

negligible relative to that of the yolk (Mine, 1995). Protein-lipid interactions typically have a negative effect on protein isolate yield and functionality, as they act to reduce protein solubility. It is likely that all isolates were spray-dried to yield a free-flowing powder, as it is the most economical drying approach for powdered ingredients. However, the high temperatures (albeit short times) employed in spray-drying can negatively affect protein functionality by inducing partial or complete denaturation (Onwulata et al., 2004). In this process, the tertiary and quaternary structures begin to unfold as hydrogen bonds break, exposing reactive groups (Kinsella, 1979; Mine, 1995).

The solubility of all protein isolates was tested at pH 7.0. WPI exhibited the highest solubility, followed by EPI (Table 4.19). The high solubility of WPI and EPI might be due to their small molecular masses relative to those of the plant proteins. For instance, α-lactalbumin in whey has a molecular mass of ~14 kDa (Kinsella & Whitehead, 1989), whereas legumin in pea has a molecular mass of 300-400 kDa (Can Karaca et al., 2011). The PPI product displayed excellent solubility at pH 7.0 versus the other plant proteins, most likely because it underwent a much milder extraction (agitation) and drying (freeze-drying) process, thus limiting damage to structure and functionality. The two soy protein isolates – SPI 974 and SPI 221 – displayed lower solubility, followed by the commercial PPIc product (Table 4.19). Low solubility in PPIc suggests high levels of denaturation during manufacturing, especially since the peas used in its preparation would have been grown in a similar region as those used to prepare the PPI product. As expected, the solubility of WhPI was very low, since the water-soluble protein content of wheat is minor in comparison to the content of prolamin-type proteins. Solubility of protein relies on the balance of protein-protein and protein-water interactions, where the latter must be favoured if proteins are to remain in solution (Hall, 1996).

Proteins bind fat via the nonpolar side chains of amino acids (Wong & Kitts, 2003). Accordingly, a sample having a higher lipid content would already have formed a number of protein-lipid complexes with its available nonpolar side chains, thereby reducing the number of sites remaining to bind oil. This would explain why high OHC was found for SPI 221 and SPI 974, which possessed low lipid contents, whereas the high lipid content of PPIc inhibited its OHC potential. The WhPI displayed moderate OHC, due to its having a low lipid content in addition to a significant number of oil binding sites, i.e. over 35% hydrophobic amino acids (Walter, 2013). Because fat absorption is a process of physical entrapment, samples that possess a lower bulk

density (higher porosity) are more proficient at binding fat (Zayas, 1997). This would explain why PPI – the only material not dried by spray-drying – might have a high OHC despite having a relatively high lipid content compared to the soy protein isolates. The PPI was freeze-dried, which would result in a highly porous material which, when mechanically crushed by hand into a coarse powder, would span a wide range of particle sizes. The low OHCs of WPI and EPI might be a consequence of their low molecular masses, where the smaller proteins possess fewer nonpolar side chains and were therefore less capable of physically entrapping oil.

Table 4.19. Functional properties of six commercial protein isolates (n = 3) and pea protein isolates prepared at laboratory scale from six pea cultivars (n = 144). Superscripts within a column denote significant differences between materials ($p < 0.05$). Data represent the mean ± one standard deviation.

Material	Sol (%)	OHC (g/g)	FC (%)	FS (%)	ES (%)
Soy, Prolisse ISE-221 (SPI 221)	28.1 ± 0.3^{d}	3.6 ± 0.2^{a}	246.7 ± 8.8^{ab}	76.5 ± 4.2^{ab}	94.3 ± 0.6^{a}
Soy, PRO-FAM 974® (SPI 974)	45.0 ± 1.3^{c}	2.3 ± 0.2^{ab}	198.9 ± 15.8^{bc}	62.7 ± 3.1^{c}	94.7 ± 1.2^{a}
Whey (WPI)	94.2 ± 4.2^{a}	1.9 ± 0.1^{b}	302.2 ± 13.9^{a}	78.7 ± 1.9^{ab}	89.3 ± 1.2^{b}
Pea (PPIc)	11.2 ± 0.2^{e}	1.5 ± 0.0^{b}	165.6 ± 31.0^{c}	56.6 ± 4.5^{d}	79.3 ± 1.2^{c}
Wheat (WhPI)	8.4 ± 0.7^{e}	2.6 ± 0.1^{ab}	192.2 ± 3.8^{bc}	9.8 ± 0.9^{e}	63.3 ± 1.2^{d}
Egg (EPI)	88.5 ± 3.8^{a}	2.1 ± 0.0^{b}	234.4 ± 20.1^{b}	79.8 ± 4.2^{a}	91.3 ± 1.2^{b}
Pea protein isolates (PPI)[1]	68.7 ± 3.5^{b}	3.2 ± 0.6^{a}	205.0 ± 27.4^{bc}	74.7 ± 2.3^{b}	95.7 ± 1.2^{a}

[1] Derived from all PPI values combining cultivars (n = 144)

Abbreviations: Sol, solubility; OHC, oil holding capacity; FC, foaming capacity; FS, foaming stability; ES, emulsion stability

The WPI (~302%) displayed higher FC than any of the other isolates, although the FC of SPI 221 was similar at ~247%. The FCs of SPI 221, SPI 974, WhPI, EPI, and PPI ranged from ~192% to 247%, followed by PPIc at ~166%. The ease of foam formation is governed by the availability and affinity of protein for the air/water interface (Wierenga and Gruppen, 2010). During foam formation, proteins migrate to the interface, unfold, re-orient, and then form an interfacial film or lamellae around gas bubbles to protect the foam from drivers of instability, such as Oswald ripening. It is hypothesized that WPI was more effective at foam formation because it was able to migrate quickly to the interface due to its high solubility (94%), as well as rearrange to stabilize the film (Zayas, 1997), suggesting that whey proteins are more flexible and surface

active than proteins in other isolates. The small nature of whey proteins would also be beneficial in terms of forming a more compact film around gas bubbles. The high total lipid content of PPIc may hinder the ability of proteins to stabilize the air/water interface, as both the lipid and protein compete for interfacial space. Lipid is known to compete favorably with protein for the air/water interface, but is unable to withstand the pressure of foam formation, thus limiting its FC (Damodaran et al., 2008). The FC of PPIc is further confounded by its low solubility which would restrict protein availability at the interface.

Foam stability is promoted by the formation of a thick and cohesive, yet flexible, film at the air/water interface (Damodaran et al., 2008; Hall, 1996). Such properties are contingent on strong intermolecular interactions, as well as short distances between adsorbed proteins (Wierenga and Gruppen, 2010; Wong & Kitts, 2003). Accordingly, the small protein sizes of EPI and WPI might have permitted close packing of proteins to produce a compact film, yet retaining the flexibility to react to stresses, resulting in high FS. Meanwhile, a more ordered native structure, such as in the globulin proteins of soy and pea, also is conducive to the formation of dense films (Dagorn-Scaviner et al., 1987). Similar FS values were observed for SPI 221 and PPI, whereas lower FS was determined for SPI 974. Although no significant correlation between Lg/Vn ratio and FS was detected as per Section 4.3.4, it is possible that the ratio of glycinin to β-conglycinin in soy has an effect on FS. From the ash content, SPI 221 was proposed to comprise greater glycinin content than SPI 974. In Section 4.2.2, it was surmised that the 11S fraction of pea likely possessed greater surface hydrophobicity than the 7S fraction. Therefore, it is logical for the 11S fraction of soy to also exhibit greater hydrophobicity and foster strong interactions amongst proteins at the interface. PPIc, despite being composed of globulins, displayed lower FS than PPI and the soy protein ingredients. This could be attributed to the high total lipid content of PPIc, where the surface-active phospholipids act to displace proteins at the interface, inhibiting their ability to form a cohesive film (Damodaran, 2005). The total lipid content of PPIc was about 2.4 times higher than that measured in PPI. Lastly, WhPI exhibited low FS. Although wheat proteins are large and highly hydrophobic in nature, they comprise 6-12% proline which has a bulky side chain which might have hindered protein flexibility and compressibility and therefore the formation of a robust film (Walter, 2013). In addition, the low FS of WhPI was confounded by its low solubility. Overall, foam stability increased with solubility because the degree of protein

solubility dictates the amount of protein available for film formation, as well as produces a more viscous solution that can reduce the rate of drainage (Yang et al., 2009).

Of the protein ingredients tested, the highest ES values were found for PPI, SPI 221, and SPI 974. This is likely the result of steric hindrance, where the large globulin proteins in soy and pea were able to form tails and loops that protrude into the aqueous phase and physically repel oil droplets from coalescing (Damodaran, 2005). Both EPI and WPI also exhibited relatively high ES. In addition to steric hindrance, natural phase separation can be counteracted by the formation of a thick and cohesive film around oil droplets, and a viscous continuous phase. The small molecular masses of egg and whey proteins afforded the flexibility required to produce compact, viscoelastic films, as well as quick rearrangement in response to stresses (Hall, 1996). Lower ES was observed for PPIc, followed by WhPI. The low solubility of PPIc and WhPI was likely one factor limiting ES. Although PPIc also comprises globulin proteins, its significantly reduced ES relative to PPI is likely attributed to its higher total lipid content. Similar to the performance of PPIc with respect to FS, pea protein adsorption to the O/W interface might have been hindered by the more surface active phospholipids, which are unable to form a cohesive film and therefore are less effective in stabilizing emulsions (Damodaran, 2005). Meanwhile, adequate steric hindrance was expected from WhPI because of its large molecular mass, but ES might have been compromised by the inability of wheat protein to form a tightly packed film due to its high proportion of bulky proline, as well as its low solubility. The stabilizing ability of WhPI is relatively high in an O/W system as compared to an air/water system, because proteins are more attracted to the higher hydrophobicity of oil as compared to air (Wierenga & Gruppen, 2010).

5. SUMMARY AND CONCLUSIONS

This study investigated the differences in, and relationships between, the physicochemical and functional properties of pea protein isolates produced from six cultivars and grown in four environments (two locations x two years). The alkaline extraction/isoelectric precipitation procedure employed was consistent in producing isolates with high protein content (~91%, d.b.) and recovery (~72%, d.b.), and did not substantially favour legumin or vicilin proteins. The Lg/Vn ratios of protein isolates were significantly different between cultivars and ranged from 0.36 (Agassiz) to 0.79 (CDC Golden), although such differences were not reflected in their amino acid compositions. Pea protein isolates were high in glutamic acid, aspartic acid, arginine, lysine, and leucine, but deficient in cysteine, methionine, and tryptophan.

Of the surface and functional properties tested, cultivars differed only in H_0, solubility, and FC. Incidentally, these were also the only properties where significant cultivar × environment interactions were found. Neither cultivar nor environment had a significant impact on ZP, OHC, FS, or ES. The four environments mainly differed in precipitation, where pea grown at the Rosthern location received considerably more rain than at Saskatoon, and pea grown in 2012 also received more rain than in 2011 at both locations. However, no noticeable trends between location, year, and cultivars could be distinguished for any property tested. The use of a much larger sample size sourced from multiple environments might be able to overcome environmental effects on cultivars, as suggested by provincial testing data of 1000-seed weights.

Correlations amongst all properties tested were generally weak, with the exception of moderately strong relationships between Lg/Vn ratio and H_0 ($r = 0.63$), and between FC and FS ($r = 0.63$). Whereas the Lg/Vn ratio was correlated only to the functional properties of solubility and ES, H_0 was significantly correlated to solubility, OHC, FC, and FS. From a structure-function approach, this suggests that altering the H_0 of pea protein isolates might have a greater impact on functional behaviours than sourcing pea cultivars with distinct Lg/Vn compositions.

From comparisons made between commercial protein isolates, raw material source was important to functionality in terms of how the flexibility and mobility of a protein were likely affected by its molecular mass and amino acid composition. However, because proteins were likely extracted using different methods, the method of protein isolate production might be the most influential factor on functional behaviour. This was most apparent in how the lack of a fat removal process in PPIc acted to hinder its solubility and other functional properties as a result. However, the inclusion of a defatting process as part of protein isolate production would increase labour, time, and cost for producers.

In addressing the hypotheses posed at the beginning of this study, the impact of cultivar and environment on physicochemical and functional properties might be mitigated by increasing the number of samples analyzed or by altering H_0 through exposure of hydrophobic residues (via protein denaturation achieved by heating or a change in pH, for example). In addition, adjustments made to the protein extraction process should modify physicochemical characteristics, which in turn would affect functional properties.

Overall, findings indicate that the effect of cultivar and growing environment on functional characteristics of pea protein isolates are secondary to those imparted by processing. Producers of pea protein isolate ingredients should find it especially beneficial that cultivars can be used interchangeably without affecting functional behaviour.

In the present study, as well as in other research on functional properties of proteins as food ingredients, the overarching restriction is in the lack of standards in functionality testing and the reporting of results. This greatly limits meaningful comparisons between works by different authors. Stemming from the conclusions of this study, future research should focus on:

a) Implementation and verification of functionality testing standards;
b) Investigating if altering the protein extraction method could overcome extreme differences in the Lg/Vn ratio and the growing environments of pea in terms of their effect on functional properties;
c) Reducing waste during pea protein isolate production, such as by co-extracting for non-globulin proteins; and
d) Studying the feasibility (cost, functionality, acceptability with respect to colour and flavour, etc.) of using pea protein isolates in commercial food production in comparison to protein isolates produced from other raw materials.

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